

Acta Obstetricia et Gynecologica Scandinavica

Editor

L. JOHANSSON Umeå

Editorial Board

D. TROLLE Copenhagen

L. RAUVAHO Turku

C. SVÄRDAL Reykjavik

O. KOLLER Bergen

. 57 1978 No 1

Edited by

SCANDINAVIAN ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

by

QVIST & WIKSELL PERIODICAL COMPANY Stockholm, Sweden

Acta Obstetricia et Gynecologica Scandinavica

Official organ of the Scandinavian Association of Obstetricians and Gynecologists

Acta Obstetricia et Gynecologica Scandinavica

publishes original papers and articles on clinical and experimental research work in the fields of obstetrics and gynecology. It brings you the best up to date information on developments and recent progress in these medical fields in the Northern countries.

The articles are published in English. Denmark, Finland, Iceland, Norway and Sweden are represented on the Editorial Board, the Swedish representative being Editor in Chief.

Subscriptions

Acta Obstetricia et Gynecologica Scandinavica is published five times annually, each issue containing towards 100 pages. Five issues constitute one volume.

per volume Sw kr 220 incl postage

Swedish subscribers Add V.A.T.

Supplements free

Editorial Office

Department of Obstetrics and Gynecology
Umeå University Hospital
S 901 85 Umeå Sweden

Editor

Professor Ingemar Joelsson

Administrative Editor

Ms Agneta Dahlquist

Subscription and Circulation

The Almqvist & Wiksell Periodical Company
P.O. Box 62
S-101 20 Stockholm 1, Sweden
Postal giro account 50464 7

Printers

Almqvist & Wiksell Tryckeri AB
S 751 81 Uppsala Sweden

Acta Obstetricia et Gynecologica Scandinavica

Published by

The Nordic Association for Obstetrics and Gynecology

Editor

Professor Ingemar Joelsson

Address

Box 443 S-901 09 Umeå Sweden

Vol 57, 1978

JR ROBERT HEILIG LIBRARY

511 Medical Center

Jalpur

N 12884

1978 10 6 92

...



Contents

<i>Beckman Gunhild Beckman L and Lofstrand T.</i> Acid and alkaline phosphatase in amniotic fluid in normal and complicated pregnancy	1
<i>Toth D J Cederqvist L L Zervoudakis A and Fuchs F.</i> Organization of amniocentesis for antenatal genetic diagnosis	7
<i>Wood C.</i> Diagnostic and therapeutic Implications of intrapartum fetal pH measurements	13
<i>Neme B and Behle I.</i> Perinatal mortality in hypertensive pregnant patients. Its reduction in a developing country	19
<i>Bleniarz J Shah N Dmowski W P Rao R and Scommegna A.</i> Premature labor treatment with Ritodrine in multiple pregnancy with three or more fetuses	25
<i>Dingfelder J R and Brenner W E.</i> The thermogenic activity of exogenous E and F prostaglandins in humans	35
<i>Meller B R Hansen J T Diederich P and Oram V.</i> Therapeutic abortion in an out patient clinic	41
<i>Thorbert G Alm P and Rosengren E.</i> Cyclic and steroid induced changes in adrenergic neurotransmitter level of Guinea pig uterus	45
<i>Öbrink A Bunne G Ulmsten U and Ingelman-Sundberg A.</i> Urethral pressure profile before during and after pubococcygeal repair for stress incontinence	49
<i>Lindstrom K and Ulmsten U.</i> Some methodological aspects on the measurement of intraluminal pressure in the female urogenital tract in vivo	63
<i>Václavíková Vlasta Hedman Anne Kristina and Nasjell Karen</i> Follow up studies in dysplasia and cancer in situ of the cervix uteri	69
<i>Husslein H Breitenacker G and Tatra G.</i> Premalignant and malignant uterine changes in immunosuppressed renal transplant recipients	73
<i>Brohult Astrid Brohult J and Brohult S</i> Regression of tumour growth after administration of alkoxyglycerols	79
<i>Kalpaktsoglou P K Ioannidou G B Kondyl A P, Lekou S I Papaconstantinou D and Comninos A C.</i> Immunochemotherapy in adenocarcinoma of the ovary	85
<i>Salvatore C A and Lodovici O.</i> Vaginal agenesis. An analysis of ninety cases	89

Short communication

<i>Novak F Kos L and Plesko F</i> The advantages of the artificial vagina derived from sigmoid colon	95
Announcement	84

Appendix

Suppl 71 Myometrial energy metabolism during pregnancy and normal dysfunctional labor By Maija Makkonen	
---	--

Acta Obstetricia et Gynecologica Scandinavica

Submission of papers

Papers should be sent in duplicate

Danish Finnish Icelandic Norwegian and Swedish contributors should send their manuscripts to Professor D Trolle Righospitalet, DK 2100 Copenhagen Professor L Rauramo Department of Obstetrics and Gynecology University Hospital Turku Dr Med G Snædal Department of Obstetrics and Gynecology University Hospital Reykjavik, Professor O Koller Kvinneklínikken Bergen and Professor I Joelsson Department of Obstetrics and Gynecology University Hospital S 901 85 Umeå respectively Contributions by foreign authors should be addressed to the Editor in Sweden

Submission of a manuscript is held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Language

Papers will be published in English. All articles should have an abstract in English not exceeding 250 words.

Arrangement

Manuscripts should be in their final form when submitted. They should be typewritten with double spacing and a wide left hand margin. The first page should contain title of paper (sub title if wanted) running headline (ca 40 characters) author's name author's department, institution or hospital location country.

The main title should contain the words necessary for correct indexing.

All titles should be as brief as clarity permits.

The text should open with an abstract which should contain a review with indications of, for example, size of material, method, new discoveries and important conclusions.

An attempt should be made to avoid footnotes. It is preferred that their contents are incorporated in the

are not bound to any particular form.
subject matter should be clearly arranged
suitable headings and sub-headings. The
following headings are preferable and in this order:
Abstract, Introduction, Methods, Material, Results,
Discussion, Acknowledgements, References.

Type sizes

The Abstract, Methods, Material, Case Reports and Acknowledgements will usually be printed in small type; these sections should be marked by the author with a blue pencil in the left hand margin. In the choice of type sizes, however, as in other typographical questions, the Editor's decision will be final.

Illustrations

Illustrations should be unmounted. The figure number (arabic numerals), author's name and title of paper should be marked in pencil on the back of every illustration. Legends of illustrations should be written on a separate sheet.

Photographs should be submitted as glossy prints with all superfluous edges cut away. The same applies to X-ray photographs, temperature graphs, etc.

Diagrams and sketches should be suitable for direct reproduction.

Since illustrations are usually reduced from the size submitted, authors should ensure that numerical and alphabetical notations within illustrations are large enough to be fully legible after reduction.

Tables

Tables should be numbered (roman numerals) each supplied with a heading and written on a separate sheet. Tables can be set to single or double-column width. They can also be set horizontally.

Figures and tables mentioned in the text should be referred to as "Fig 1" and "Table VII" etc. The approximate position of figures and tables should be indicated in the margin.

Pharmaceutical preparations

Pharmaceutical preparations should always be mentioned in the text by their generic names, with trade name and manufacturer's name in parentheses.

References

The reference list—containing only necessary references—should be arranged alphabetically, each reference being numbered. Abbreviations (no points). *Index Medicus* Examples

- 1 Cruickshank, B & Stuart Smith D A. Orchitis associated with spermagglutinating antibodies. *Lancet* 1 708 1959
- 2 Fjellbrant, B. Immunosagglutination of sperm in cases of sterility. *Acta Obstet Gynecol Scand* 44 474 1965
- 3 Mann T. The Biochemistry of Semen and of the Male Reproductive Tract. Methuen, London, 1964
- 4 Snyder F F & Hoskins F M. In A Textbook of Obstetrics (ed D E Reld) p 81 Saunders Philadelphia and London 1962.

When several papers by the same author are quoted, the papers should be numbered in chronological order.

Text references should be indicated by figures within parentheses. Examples: the incidence is similar to that in other reports (1 5 11 17) Davies et al (6) have reported.

General

Authors are requested in the interest of all concerned to restrict the length of papers as much as possible. Papers should not exceed 16 printed pages including tables and illustrations. Exceptionally 24 pages can be allowed by the chief editor if a paper exceeds 12 printed pages (about 30 typewritten sheets), the cost for the excess pages as well as costs for tables and illustrations exceeding Sw kr 300 will be borne by the author.

Illustrations may be printed in colour if so requested, but at the author's expense.

Manuscripts exceeding 24 printed pages can be published as a supplement at the author's expense. Short reports of at most two printed pages will receive priority and be published as soon as possible.

Cost of corrections in the proofs against the manuscript will be defrayed by the author. Published manuscripts are not returned unless requested.

Proofs and reprints

The printer's proof (together with an order form) will be sent to the principal author by the Editorial Office. It should be returned to the secretary without delay. Reprints of published articles should be ordered simultaneously.

Contents

<i>echer N J and Wallin L</i> Haemodynamic effects of oxytocin (Syntocinon®) and methyl ergometrine (Methergin®) on the systemic and pulmonary circulations of pregnant anaesthetized women	97
<i>Vallenburg H C., van Kessel P H and Brand Anneke</i> Transfer of ⁵¹ Cr platelets and ⁵¹ Chromium ions across the term rhesus monkey placenta	105
<i>Jeyth Y</i> Improved method for hystero-graphic evaluation of uterine scar	111
<i>Åxelsson O., Lindberg B S., Nilsson, B A. and Johansson E. D B</i> Plasma levels of non conjugated oestrone in high risk pregnancies	113
<i>Coats P., Florensa E., Youssefnejadian E and Craft, I</i> Plasma steroids in the foetal and maternal circulation at normal delivery and elective caesarean section	121
<i>Štulc J., Švihovec J., Drábková J., Strbímy J., Kobilková J., Vido I and Dolezal A.</i> Electrical potential difference across the mid term human placenta	125
<i>Bergqvist, G., Holmberg G., Rydner T and Václavíková, Vlasta</i> Intrauterine death due to infection with group B streptococci	127
<i>Fianu S</i> Maternal mortality in Sweden 1955-1974	129
<i>Møller B R., Hansen J T and Mømmesen S</i> Effect of general and local anaesthesia on blood loss during and after therapeutic abortion	133
<i>Gjønnaess H and Holten E</i> Doxycycline (Vibramycin®) in pelvic inflammatory disease	137
<i>Martin N J., Jr Bygdeman M and Eneroth P</i> The influence of locally administered prostaglandin E ₁ and F _{2α} on uterine motility in the intact non pregnant human uterus	141
<i>Kullander S., Rausing A. and Tropé C</i> Human ovarian tumours heterotransplanted to "nude" mice	149
<i>Welander C., Kjerstad K E and Kolstad P</i> Postoperative irradiation and chemotherapy in patients with advanced ovarian cancer	161
<i>Frick, G., Johnsson J E., Landberg T and Snorrðóttir M</i> Relaparotomy in advanced ovarian carcinoma	165
<i>Gregersen E. and Kjer J J</i> Bipolar cautery for laparoscopic sterilization	169
<i>Keskarela D B</i> An abdominal approach to the surgical repair of post hysterectomy vaginal inversion	173
Case Reports	
<i>Sadovsky E and Perlman, M</i> Decreased fetal movements and polyhydramnios	177
<i>Moldin P and Johansson O</i> Acute fatty liver of pregnancy with disseminated intra vascular coagulation	179
<i>Hansson U., Irestedt L. and Moberg P. J</i> Delivery complicated by myasthenia gravis and epilepsy	183
<i>Christau Susanne and Klebe J G</i> Rupture of the spleen during delivery	187
<i>Hammar M and Larsson Cohn U</i> Massive enlargement of occluded tubes after postmenopausal treatment with natural estrogens	189
Letter to the Editor	
<i>Maltau J M and Andersen H T</i> On "the use of epidural anesthesia in obstetrics" <i>Acta Obstet Gynecol Scand</i> 55 469-470 1976	191
Announcements	192

Appended

Suppl 72. Aspects of placental pathology and growth retardation A prospective pathological anatomical and cellular study By John Rolschau

Acta Obstetricia et Gynecologica Scandinavica

Submission of papers

Papers should be sent in duplicate

Danish Finnish Icelandic Norwegian and Swedish contributors should send their manuscripts to Professor D. Trolle Rigshospitalet, DK 2100 Copenhagen Professor L. Rauramo Department of Obstetrics and Gynecology University Hospital Turku Dr Med G. Snødal Department of Obstetrics and Gynecology University Hospital Reykjavik Professor O. Koller Kvinneklínikken Bergen and Professor I. Joelsson, Department of Obstetrics and Gynecology University Hospital S 901 85 Umeå respectively Contributions by foreign authors should be addressed to the Editor in Sweden

Submission of a manuscript is held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Language

Papers will be published in English. All articles should have an abstract in English not exceeding 250 words.

Arrangement

Manuscripts should be in their final form when submitted. They should be typewritten with double spacing and a wide left hand margin. The first page should contain title of paper (sub title if wanted) running headline (ca 40 characters) author's name, author's department, institution or hospital, location, country.

The main title should contain the words necessary for correct indexing.

All titles should be as brief as clarity permits.

The text should open with an abstract which should contain a review with indications of for example size of material, method, new discoveries, and important conclusions.

An attempt should be made to avoid footnotes. It is preferred that their contents are incorporated in the

text and are not bound to any particular form.

Subject matter should be clearly arranged in headings and sub headings. The

following headings are preferable and in this order:

Abstract, Introduction, Methods, Material, Results, Discussion, Acknowledgements, References.

Type sizes

The Abstract, Methods, Material, Case Reports and Acknowledgements will usually be printed in small type. These sections should be marked by the author with a blue pencil in the left hand margin. In the choice of type sizes, however, as in other typographical questions, the Editor's decision will be final.

Illustrations

Illustrations should be unmounted. The figure number (arabic numerals), author's name and title of paper should be marked in pencil on the back of every illustration. Legends of illustrations should be written on a separate sheet.

Photographs should be submitted as glossy prints with all superfluous edges cut away. The same applies to X ray photographs, temperature graphs, etc.

Diagrams and sketches should be suitable for direct reproduction.

Since illustrations are usually reduced from the size submitted, authors should ensure that numerical and alphabetical notations within illustrations are large enough to be fully legible after reduction.

Tables

Tables should be numbered (roman numerals) each supplied with a heading and written on a separate sheet. Tables can be set to single or double-column width. They can also be set horizontally.

Figures and tables mentioned in the text should be referred to as "Fig 1" and "Table VII" etc. The approximate position of figures and tables should be indicated in the margin.

Pharmaceutical preparations

Pharmaceutical preparations should always be mentioned in the text by their *generic names* with trade name and manufacturer's name in parentheses.

References

The reference list—containing only necessary references—should be arranged alphabetically, each reference being numbered. Abbreviations (no points) *Index Medicus* Examples:

1. Cruickshank, B. & Stuart Smith, D. A. Orchids associated with spermagglutinating antibodies. *Lancet* 1, 708, 1959.
2. Fjellbrant, B., Immunoagglutination of sperm in cases of sterility. *Acta Obstet Gynecol Scand* 44, 474, 1965.
3. Mann, T., The Biochemistry of Semen and of the Male Reproductive Tract. Methuen, London, 1964.
4. Snyder, F. F. & Hoskins, F. M., *In A Textbook of Obstetrics* (ed D. E. Reid) p. 81 Saunders, Philadelphia and London, 1962.

When several papers by the same author are quoted, the papers should be numbered in chronological order.

Text references should be indicated by figures within parentheses. Examples: the incidence is similar to that in other reports (1, 5, 11, 17). Davies et al. (6) have reported.

General

Authors are requested in the interest of all concerned to restrict the length of papers as much as possible. Papers should not exceed 16 printed pages including tables and illustrations. Exceptionally 24 pages can be allowed by the chief editor. If a paper exceeds 12 printed pages (about 30 typewritten sheets), the cost for the excess pages as well as costs for tables and illustrations exceeding Sw. kr. 300 will be borne by the author.

Illustrations may be printed in colour if so requested, but at the author's expense.

Manuscripts exceeding 24 printed pages can be published as a supplement at the author's expense.

Short reports of at most two printed pages will receive priority and be published as soon as possible.

Cost of corrections in the proofs against the manuscript will be defrayed by the author. Published manuscripts are not returned unless requested.

Proofs and reprints

The printer's proof (together with an order form) will be sent to the principal author by the Editorial Office. It should be returned to the secretary without delay. Reprints of published articles should be ordered simultaneously.

ontents

<i>Andersen S</i> An electroimmuno assay of the pregnancy specific β_1 glycoprotein (PS ₁) in normal and pathological pregnancies and its clinical value compared to human chorionic somato mammatropin (HCS)	193
<i>Andersson P, Trolldenier D and Pedersen H</i> Extremely low placental lactogen hormone (PLH) values in an otherwise uneventful pregnancy preceding delivery of a normal baby	203
<i>Andersson G</i> The value of serum cystine aminopeptidase (CAP) human chorionic somatomammatrophin (HCS) and urinary oestrogen assays for detecting intrauterine growth retardation	211
<i>Andersson B and Hedner T</i> Antepartum administration of terbutaline and the incidence of hyaline membrane disease in preterm infants	217
<i>Andersson G and Katz M</i> Midtrimester intra amniotic administration of prostaglandin E_2 in combination with an hyperosmolar urea solution. Effect upon plasma levels of estradiol progesterone and human placental lactogen (HPL)	223
<i>Andersson Larsen J., Jacobsen B., Holm H H, Fog Pedersen J and Mantoni Margit</i> Intravenous injection of vitamin K before the delivery during anticoagulant therapy of the mother	227
<i>Andersson H</i> Changes in fetal supraventricular extrasystoles during uterine contractions in labour	231
<i>Andersson F and Fylling P</i> Therapeutic abortion—The 1975 report from Ullevål Hospital	237
<i>Andersson R S, Hagman E and Pystynen P</i> Effect of fasting on blood glucose of parturient and her full term infant	241
<i>Andersson Eva and Larsson B</i> Caesarean section. A clinical study with special reference to the increasing section rate	245
<i>Andersson J., Kaar K., Jouppila P., Pyörälä T and Rekonen A.</i> An intravenous ^{133}Xe method for measuring regional distribution of placental blood flow	249
<i>Andersson Inger</i> Ultrastructure of human umbilical veins	253
<i>Andersson B and Rybo G</i> Conjoined twinning in Sweden	257
<i>Andersson L G., Kullander S., Persson J P and Korsten C B</i> On receptors for oestrogens (E_2) and androgens (DHT) in human endometrial carcinoma and ovarian tumours	261
<i>Andersson K., Lash A. F and Webster Augusta</i> Pregnancy and sarcoma	265
<i>Andersson B, Bar Am A and Deligdisch Liane</i> Cervical adeno carcinoma and partial hydatidiform mole	273
<i>Andersson S and Taina E</i> Operative treatment of rectal endometriosis	277
<i>Andersson B</i> Treatment of menopausal oestrogen deficiency symptoms in hysterectomised women by means of 17β oestradiol pellet implants	281
Short communication	
<i>Andersson O., Nygren K G., Fagerlund Christina and Hartvig P</i> Absorption of a 8 hydroxyl quinoline (Stereosan) through the vaginal mucosa	287
Case Report	
<i>Andersson L., Carlström K and Eriksson Margareta</i> Hypervitaminosis A in early human pregnancy and malformations of the central nervous system	289
Announcements	292
Appendix	

Acta Obstetricia et Gynecologica Scandinavica

Submission of papers

Papers must be sent in duplicate

Danish Finnish Icelandic Norwegian and Swedish contributors should send their manuscripts to Professor O Trolle Rigshospitalet, DK 2100 Copenhagen Professor L Rauramo Department of Obstetrics and Gynecology University Hospital Turku Dr Med G Snædal Department of Obstetrics and Gynecology University Hospital Reykjavik Professor O Koller Kvinnekliviken Bergen and Professor I Joelsson Department of Obstetrics and Gynecology University Hospital S 901 115 Umeå respectively Contributions by foreign authors should be addressed to the Editor in Sweden

Submission of a manuscript is held to imply that the paper has not been published elsewhere and that if accepted it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Language

Papers will be published in English All articles should have an abstract in English not exceeding 250 words

Arrangement

Manuscripts should be in their final form when submitted They should be typewritten with double spacing and a wide left hand margin The first page should contain title of paper (sub title if wanted) running headline (ca 40 characters) author's name author's department, institution or hospital location country

The main title should contain the words necessary for correct indexing

All titles should be as brief as clarity permits

The text should open with an abstract which should contain a review with indications of for example size of material method new discoveries and important conclusions

An attempt should be made to avoid footnotes It is preferred that their contents are incorporated in the text.

Authors are not bound in any particular form However subject matter should be clearly arranged with suitable headings and sub headings The following headings are preferable and in this order: Abstract Introduction Methods Material Results Discussion Acknowledgements References

Type sizes

The Abstract, Methods, Material, Case Reports and Acknowledgements will usually be printed in small type these sections should be marked by the author with a blue pencil in the left hand margin In the choice of type sizes however as in other typographical questions the Editor's decision will be final

Illustrations

Illustrations should be unmounted The figure number (arabic numerals) author's name and title of paper should be marked in pencil on the back of every illustration Legends of illustrations should be written on a separate sheet.

Photographs should be submitted as glossy prints with all superfluous edges cut away The same applies to X ray photographs temperature graphs etc.

Diagrams and sketches should be suitable for direct reproduction

Since illustrations are usually reduced from the size submitted authors should ensure that numbers and alphabetical notations within illustrations are large enough to be fully legible after reduction.

Tables

Tables should be numbered (roman numerals) and supplied with a heading and written on a separate sheet. Tables can be set to single or double width They can also be set horizontally

Figures and tables mentioned in the text referred to as "Fig 1" and "Table VII" etc. The approximate position of figures and tables indicated in the margin

Pharmaceutical preparations

Pharmaceutical preparations should always be mentioned in the text by their generic names with trade name and manufacturer's name in parentheses

References

The reference list—containing only necessary references—should be arranged alphabetically and reference being numbered Abbreviations (no *Index Medicus* Examples:

- 1 Cruickshank, B & Stuart Smith D A. Orchitis associated with spermagglutinating antibodies. *Lancet* 1 708 1959
- 2 Fjellbrant, E. Immunoagglutination of sperm in cases of sterility *Acta Obstet Gynecol Scand* 44 474 1965
- 3 Mann T. The Biochemistry of Semen and of Male Reproductive Tract. Methuen, London, 1941
- 4 Snyder F F & Hoskins F M. In A Textbook of Obstetrics (ed D E. Reid) p 81 Saunders, Philadelphia and London, 1962.

When several papers by the same author are quoted the papers should be numbered in chronological order

Text references should be indicated by figures within parentheses Examples the incidence is similar to that in other reports (1 5 11 17) Dark et al (6) have reported

General

Authors are requested in the interest of all concerned to restrict the length of papers as much as possible Papers should not exceed 16 printed pages including tables and illustrations. Except allowed by the chief editor if a paper exceeds 12 printed pages (about 30 typewritten sheets) for the excess pages as well as costs for tables and illustrations exceeding Sw kr 300 will be borne by the author

Illustrations may be printed in colour if so requested but at the author's expense

Manuscripts exceeding 24 printed pages can be published as a supplement at the author's expense

Short reports of at most two printed pages will receive priority and be published as soon as possible

Cost of corrections in the proofs against the manuscript will be defrayed by the author Published manuscripts are not returned unless requested.

Proofs and reprints

The printer's proof (together with an order form) will be sent to the principal author by the Editorial Office It should be returned to the secretary without delay Reprints of published articles should be ordered simultaneously

Contents

<i>Okano R, Hashiba N, Washio M and Tojo S</i>	Ovarian follicular apparatus and parameters in patients with primary and secondary amenorrhea	293
<i>K and Mroueh A</i>	Ovarian biopsy in the evaluation of amenorrhea	301
<i>Ydén B and Berg G</i>	CAP HCS and urinary oestrol assays in diabetic pregnancy	313
<i>R E, Joelsson I and Adamsons K</i>	The effects of isoproterenol on fetal	317
<i>ergman B, Hedner T and Lundborg P</i>	Effects of terbutaline on the pressure-volume relationship in fetal rabbit lung	323
<i>agab M I, Edelman D A and Laufe L</i>	The effects of longacting paracervical block anesthesia on the abortifacient efficacy of intra amniotic PGF _{2α} and hypertonic saline	327
<i>lund A and Larsson B</i>	Comparison of extra amniotic instillation of rivanol and GF _{15a} either separately or in combination followed by oxytocin for second trimester	333
<i>hrétien F C</i>	Ultrastructure and variations of human cervical mucus during pregnancy and the menopause	337
<i>Y, Lazer S and Ben Aderet, N</i>	Fracture and chemical composition of the it formed on the Lippes loop after prolonged use	349
<i>Junne G and Öbrink, A</i>	Influence of pubococcygeal repair on urethral closure pressure at stress	355
<i>merth Y</i>	Cryosurgical treatment of dysplasia and carcinoma in situ of the cervix	361
communication		
<i>Polis D and Kaskarelis D</i>	Human placental lactogen levels in amniotic fluid in normal and toxemic pregnancies	367
<i>Janu S and Václavínková V</i>	The site of placental attachment as a factor in the etiology of breech presentation	371
Report		
<i>Feiba S, Kaufman H, Winkelsberg G and Bahary C</i>	Pregnancy in a case of Nelson's syndrome	373
<i>Ahlberg B and Ahlmark, G</i>	The Landry Guillaume Barré syndrome and pregnancy	377
<i>Patek, E and Johnson, P</i>	Recurrent hydatiform mole: Report of a case with five	381
<i>Il Czernobilsky B, Borenstein R and Lancet, M</i>	Granular cell myoblastoma of the vulva: Report of 4 cases	385
Announcements		388
Appended		
Suppl 75 Carcinoma cervicis uteri stages I and IIa. By Sture Cullhed		
Suppl 76 Influence of oral contraceptives on immediate postabortal pituitary ovarian		
ion By Pekka Lahteenmaki.		

Acta Obstetricia et Gynecologica Scandinavica

Submission of papers

Papers should be submitted as the top copy and one carbon or photostatic copy of the typewritten double spaced manuscript.

Danish, Finnish, Icelandic and Norwegian contributors should send their manuscripts to the regional editors.

Professor Dyrre Trolle, Rigshospitalet, Copenhagen, Denmark.
Professor Lauri Rauramo, The University Central Hospital, Turku, Finland.
Dr Med. Gunnlaugur Snædal, The University Hospital, Reykjavik, Iceland.
and Professor Oddmund Koller, Haukeland Sykehus, Bergen, Norway.
Contributions by Swedish and by foreign authors should be addressed to the Chief Editor, Professor Ingemar Joelsson, The Editorial Office of Acta Obstet et Gynecol Scand, Box 443, S 901 08 Umeå, Sweden.

Chief Editor

Submission of a manuscript is held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Language

Papers will be published in English. All articles should have an abstract in English not exceeding 250 words.

Arrangement

Manuscripts should be in their final form when submitted. The first page should contain title of paper (sub title if wanted), running headline (ca 40 characters), author's name, author's department, institution or hospital, location, country.

The main title should contain the words necessary for correct indexing.

All titles should be as brief as clarity permits. The text should open with an abstract which should contain a review with indications of e.g. size of material, methods, new discoveries and important conclusions.

An attempt should be made to avoid footnotes. It is preferred that their contents are incorporated in the text.

Authors are not bound to any particular form. However, subject matter should be clearly arranged with suitable headings and sub-headings. The following headings are preferable and in this order: Abstract, Introduction, Methods, Material, Results, Discussion, Acknowledgments, References.

Type sizes

The Abstract, Methods, Material, Case Reports and Acknowledgements will usually be printed in small type; these sections should be marked by the author with a blue pencil in the left hand margin in the choice of type sizes, however, as in other typographical questions, the Editor's decision will be final.

Illustrations

(Illustrations should be unmounted. The figure number (arabic numerals), author's name and title of paper should be marked in pencil on the back of every illustration. Legends of illustrations should be written on a separate sheet.

Photographs should be submitted as glossy prints with all superfluous edges cut away. The same applies to X-ray photographs, temperature graphs etc.

Diagrams and sketches should be suitable for direct reproduction.

Since illustrations are usually reduced from the size submitted, authors should ensure that nu- and alphabetical notations within illustrations are large enough to be fully legible after reduction.

Tables

Tables should be numbered (roman numerals) as supplied with a heading and written on a separate sheet. Tables can be set to single or double-column width. They can also be set horizontally.

Figures and tables mentioned in the text should be referred to as "Fig 1" and "Table VII" etc. The approximate position of figures and tables should be indicated in the margin.

Pharmaceutical preparations

Pharmaceutical preparations should always be mentioned in the text by their generic names, with trade name and manufacturer's name in parentheses.

References

The reference list—containing only necessary references—should be arranged alphabetically, each reference being numbered. Abbreviations (no *post Index Medicus* Examples.

- 1 Cruickshank, B. & Stuart Smith, D. A. Orchitis associated with spermagglutinating antibodies. *Lancet* 1, 708, 1959.
- 2 Fjällbrant, B. Immunoagglutination of sperm in cases of sterility. *Acta Obstet Gynecol Scand* 44, 474, 1965.
- 3 Mann, T. The Biochemistry of Semen and of the Male Reproductive Tract. Methuen, London, 1961.
- 4 Snyder, F. F. & Hoskins, F. M., Jr. *A Textbook of Obstetrics* (ed. D. E. Reld), p. 81. Saunders, Philadelphia and London, 1962.

When several papers by the same author are quoted, the papers should be numbered in chronological order.

Text references should be indicated by figures within parentheses. Examples: the incidence is similar to that in other reports (1, 5, 11, 17). David et al. (6) have reported.

General

Authors are requested in the interest of all concerned to restrict the length of papers as much as possible. Papers should not exceed 16 printed pages including tables and illustrations. Exceptionally, 24 pages are allowed by the chief editor if a paper exceeds 12 printed pages (about 30 typewritten sheets) then for the excess pages as well as costs for tables and illustrations exceeding Sw. kr. 300 will be borne by the author.

Illustrations may be printed in colour if so requested, but at the author's expense.

Manuscripts exceeding 24 printed pages can be published as a supplement at the author's expense. Short reports of at most two printed pages will receive priority and be published as soon as possible.

Cost of corrections in the proofs against the manuscript will be defrayed by the author. Published manuscripts are not returned unless requested.

Proofs and reprints

The printer's proof (together with an order form) will be sent to the principal author by the Editorial Office. It should be returned to the secretary without delay. Reprints of published articles should be ordered simultaneously.

Contents

mmintausta R., Erkkola R. and Eronen M. Effect of chlorthalidate treatment on aldosterone system during pregnancy	389
ndahl B. Seasonal birth pattern in Sweden in relation to birth order and maternal age	393
ochizuki M., Honda T., Deguchi M., Monkawa H. and Tojo S. A study on the effect of dehydroepiandrosterone sulfate on so called cervical ripening	397
ingerup L., Andersson K. E. and Ulmsten U. Ripening of the uterine cervix and induction of labour at term with prostaglandin E ₂ in viscous gel	403
lerius N. H. and Ramsoe Jacobsen J. Intrauterine supraventricular tachycardia	407
ackleton C. H. L., Macrae D. J. and Wilmott, M. P. Comparison of oestriol in mother and fetus during labour and in the baby at birth	411
oberg P. J., Eneroth P., Harlin J., Lyng Åsa and Nord C. E. Preoperative cervical microbial flora and post abortion infection	415
undstrom V. Treatment of primary dysmenorrhea with prostaglandin synthetase inhibitors—a promising therapeutic alternative	421
kerlund M., Bengtsson L. Ph. and Ulmsten, U. Recording of myometrial activity in the non pregnant human uterus by a microtransducer catheter	429
elén N., Furuholm M., Jacobson B. and Lemke B. Changes in bone mineral content in women with natural menopause during treatment with female sex hormones	435
deligdisch L., Yedwab G., Pelsitz, A. and David M. P. Ultrastructural features in normal and hyperplastic postmenopausal endometrium	439
faram K. and Digraes A. Vulvovaginal candidiasis in pregnancy treated with clotrimazole	453
rud T., Ulmsten U. and Andersson K. E. Initiation of voiding in healthy women and those with stress incontinence	457
Olsson K. P. and Walter S. Bladder base insufficiency	463
Beyth Y. and Laufer N. A new method for pregnancy termination in polyhydramnios	469

Short communication

Puikkinen M. O., Salminen J. and Vatanen S. Serum vitamin B ₆ in pure pregnancy depression	471
---	-----

Case Reports

Eika C., Amesen H. and Godal H. C. The ethanol gelation test in pregnancy	473
Ladehoff P. and Maruszczak M. A pregnancy with a hydatidiform mole, thyrotoxicosis and live born infant	477
Åkerlund M. Myometrial activity and endometrial blood flow in an ectopic pregnancy	479
Biale Y., Lev-ohai H., Alteras M. and Ben Aderet N. Anencephaly and clomiphene induced pregnancy	483
Announcement	482

Appendix

Suppl 77 Obstetric service and perinatal mortality in Norway By Lev S. Bakkevig Howard J. Hoffman and Phyllis M. Sternthal	
Suppl 78 Ultrasound screening in pregnancy Methodology and significance By P. H. Persson L. Grennert and G. Gennser	

Acta Obstetrica et Gynecologica Scandinavica

Submission of papers

Papers should be submitted as the top copy and one carbon or photostatic copy of the typewritten double spaced manuscript.

Danish Finnish Icelandic and Norwegian contributors should send their manuscripts to the regional editors

Professor Dyre Trolle Rigshospitalet, Copenhagen Denmark, Professor Lauri Rauramo The University Central Hospital Turku Finland Dr Med Gunnlaugur Snædal The University Hospital Reykjavik, Iceland and Professor Oddmund Koller Haukeland Sykehus Bergen Norway Contributions by Swedish and by foreign authors should be addressed to the Chief Editor Professor Ingemar Joelsson The Editorial Office of Acta Obstet et Gynecol Scand Box 443 S 901 09 Umeå Sweden

Submission of a manuscript is held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Language

Papers will be published in English. All articles should have an abstract in English not exceeding 250 words.

Arrangement

Manuscripts should be in their final form when submitted. The first page should contain title of paper (sub title if wanted) running headline (ca 40 characters) author's name author's department, institution or hospital location country.

The main title should contain the words necessary for correct indexing.

All titles should be as brief as clarity permits.

The text should open with an abstract which should contain a review with indications of e.g. size of material, methods, new discoveries and important conclusions.

An attempt should be made to avoid footnotes. It is preferred that their contents are incorporated in the text.

Authors are not bound to any particular form. However, subject matter should be clearly arranged with suitable headings and sub-headings. The following headings are preferable and in this order: Abstract, Introduction, Methods, Material, Results, Discussion, Acknowledgments, References.

Type sizes

The Abstract, Methods, Material, Case Reports and Acknowledgments will usually be printed in small type; these sections should be marked by the author with a blue pencil in the left hand margin in the choice of type sizes. However, as in other typographical questions, the Editor's decision will be final.

Illustrations

Illustrations should be unmounted. The figure number (arabic numerals), author's name and title of paper should be marked in pencil on the back of every illustration. Legends of illustrations should be written on a separate sheet.

Photographs should be submitted as glossy prints with all superfluous edges cut away. The same applies to X-ray photographs, temperature graphs, etc.

Diagrams and sketches should be suitable for direct reproduction.

Since illustrations are usually reduced from the size submitted, authors should ensure that numerical and alphabetical notations within illustrations are large enough to be fully legible after reduction.

Tables

Tables should be numbered (roman numerals) each supplied with a heading and written on a separate sheet. Tables can be set to single or double column width. They can also be set horizontally.

Figures and tables mentioned in the text should be referred to as "Fig 1" and "Table VII" etc. The approximate position of figures and tables should be indicated in the margin.

Pharmaceutical preparations

Pharmaceutical preparations should always be mentioned in the text by their generic names with trade name and manufacturer's name in parentheses.

References

The reference list—containing only necessary references—should be arranged alphabetically and reference being numbered. Abbreviations (no page numbers) *Index Medicus* Examples

1. Cruickshank, D. & Stuart Smith, D. A. Orbits associated with spermagglutinating antibodies. *Lancet* 1: 708, 1959.
2. Fjällbrant, B. Immunoagglutination of sperm in cases of sterility. *Acta Obstet Gynecol Scand* 44: 474, 1955.
3. Mann, T. The Biochemistry of Semen and of the Male Reproductive Tract. Methuen, London, 1954.
4. Snyder, F. F. & Hoskins, F. M. In: A Textbook of Obstetrics (ed. D. E. Reid) p. 811 Saunders, Philadelphia and London, 1962.

When several papers by the same author are quoted, the papers should be numbered in chronological order.

Text references should be indicated by figures within parentheses. Examples: "the incidence is similar to that in other reports (1, 11, 17). Davis et al. (6) have reported."

General

Authors are requested in the interest of all concerned to restrict the length of papers as much as possible. Papers should not exceed 16 printed pages in text, tables and illustrations. Exceptionally 24 pages are allowed by the chief editor if a paper exceeds 12 printed pages (about 30 typewritten sheets). The cost for the excess pages as well as costs for tables and illustrations exceeding Sw. kr. 300 will be borne by the author.

Illustrations may be printed in colour if so requested, but at the author's expense. Manuscripts exceeding 24 printed pages can be published as a supplement at the author's expense.

Short reports of at most two printed pages will receive priority and be published as soon as possible.

Cost of corrections in the proofs against the manuscript will be defrayed by the author. Published manuscripts are not returned unless requested.

Proofs and reprints

The printer's proof (together with an order form) will be sent to the principal author by the Editorial Office. It should be returned to the secretary without delay. Reprints of published articles should be ordered simultaneously.

ACID AND ALKALINE PHOSPHATASE IN AMNIOTIC FLUID
IN NORMAL AND COMPLICATED PREGNANCY

Gunhild Beckman Lars Beckman and Tord Löfstrand

*From the Departments of Obstetrics and Gynaecology and Medical Genetics
University of Umeå Umeå Sweden*

Abstract 171 samples of amniotic fluid were obtained by abdominal amniocentesis from 67 women with complicated pregnancies (isoimmunization, diabetes mellitus or toxæmia). The levels of heat labile alkaline phosphatase (AP), heat stable alkaline phosphatase (HSAP) and acid phosphatase (AcP) were determined and compared to the enzyme levels in 179 samples from women with normal pregnancies of corresponding gestational ages. HLAP showed two peaks of activity, one in the 15th–22nd week and the other at term. HSAP and AcP showed increased activity at term. HSAP was decreased ($p < 0.01$) in isoimmunization between the 36th and 40th week. In toxæmia with placental insufficiency, no differences in the levels of HLAP and HSAP compared to normal pregnancy. AcP showed no differences between normal and complicated pregnancy. Samples contaminated by blood showed no significant increase in the acid and alkaline phosphatase levels. Samples contaminated by meconium showed a complex pattern. Some had normal enzyme levels, some had high levels of HLAP only and some had high levels of HSAP and AcP. The origin of the enzymes is not known with certainty. HSAP in amniotic fluid is most likely not of placental but of intestinal origin. Determinations of acid and alkaline phosphatase in amniotic fluid seem to be of little value in the clinical management of complicated pregnancies.

In erythroblastosis Geyer & Schneider (5) found elevated levels of acid phosphatase (AcP) and alkaline phosphatase (AP). Roopnarinesingh et al (11) observed an increase of heat stable alkaline phosphatase (HSAP) in pre-eclampsia. Fennefrohn (3) suggested that an AP level above 10 KA units indicates fetal maturity, while >20 KA units is indicative of fetal growth retardation and placental insufficiency. Benzie et al (2) found normal values of AcP and AP in intrauterine growth retardation, diabetes, Rh immunization, hydramnios and hypertensive disorders. Several authors have attempted to determine the

origin of AcP and AP in amniotic fluid. Sutcliffe & Brock (12) suggested that at term AcP originates both from the surrounding tissues and from fetal urine. Concerning the origin of AP, there are conflicting views. The results of Sutcliffe & Brock (12) and Jonasson (7) indicate that HSAP in amniotic fluid is of placental origin. However, Kellen et al (8) and Hahnemann & Sørensen (6) claim that this is not the case.

This paper reports the results of a study on HLAP, HSAP and AcP in normal pregnancy and in pregnancy complicated by isoimmunization, diabetes mellitus or toxæmia.

MATERIAL AND METHODS

The normal material consists of 179 samples from 170 pregnant healthy subjects. 171 samples of amniotic fluid were obtained by abdominal amniocentesis from 67 women with complicated pregnancies in the 27th–43rd week. Samples containing macroscopically detectable blood or meconium were discarded; only clear fluid was accepted. Diagnostic criteria for pregnancy complications and for estimation of gestational age have been presented previously (4, 9, 10).

The amniotic fluid was centrifuged, filtered through filterpaper and stored at -18°C until enzyme analysis. AcP and AP were determined according to methods previously described (1). To identify different isoenzymes in the amniotic fluid, double-diffusion techniques and starch gel electrophoresis were used.

RESULTS

In Tables I–III, data for normal and complicated pregnancies have been divided into four periods according to gestational age. HLAP showed significant differences between consecutive periods (Table I). HSAP showed a significant increase for

Table 1 Heat labile alkaline phosphatase activity in amniotic fluid ($\mu\text{mol } \alpha\text{-naphthol}$ liberated per ml/h) in normal pregnancies and pregnancies complicated by immunization diabetes and toxæmia

Figures within brackets indicate number of samples examined Mean \pm S D

Pregnancy week	Normal pregnancy	Pregnancy complicated by		
		Immunization	Diabetes	Toxæmia
30 and earlier	1.98 \pm 1.22 (47)	0.23 \pm 0.16 (10)	—	—
31-35	0.35 \pm 0.29 (34)	0.34 \pm 0.19 (29)	0.84 \pm 2.00 (13)	0.36 \pm 0.44 (70)
36-40	1.25 \pm 1.07 (74)	1.68 \pm 5.06 (51)	0.76 \pm 0.80 (21)	2.31 \pm 4.56 (25)
41 and later	3.34 \pm 3.01 (74)	—	—	4.75 \pm 1.45 (7)

the three first periods and then a non significant decrease (Table II). AcP increased significantly from the third to the fourth period (Table III).

When comparing the enzyme levels in normal pregnancy with those in complicated pregnancy a significant decrease in HSAP activity was found in isoimmunization in the 36th-40th week ($p < 0.01$). The difference in HLAP level between normal pregnancy and isoimmunization before the 31st week was highly significant ($p < 0.001$) but trivial. Samples from pregnancies before the 27th week where the HLAP level is high were lacking among the pregnancies complicated by isoimmunization. AcP showed no difference between normal and complicated pregnancy.

The effect of contamination by blood or meconium in full term pregnancies is shown in Table IV. In samples contaminated by blood the average enzyme levels were not significantly increased. In samples macroscopically contaminated by meconium the average levels of all three enzymes were significantly increased when compared to the levels for normal pregnancy. Meconium contaminated samples however showed a complex pattern. 9 of the 23 samples showed normal activity of all three enzymes. 12 samples had high levels ($> 10 \mu\text{mol}$) of HLAP. 6 of these also had high levels

($> 5 \mu\text{mol}$) of HSAP. The remaining 2 samples showed high activity of HSAP but low levels of HLAP. The 8 samples with high levels of HSAP had high levels of AcP. Table V shows the relation between AcP and HSAP in the macroscopically contaminated samples. Thus samples with an AcP level above $0.3 \mu\text{mol}$ showed a fourfold increase of the HSAP level compared to samples with an AcP level below $0.3 \mu\text{mol}$. In this connection will be mentioned that 4 samples of amniotic fluid from normal pregnancy are not accounted for in the Tables. These 4 samples showed very high AP values 10 S.D.s above the mean of the other 29 samples of the same gestational age (weeks 39-41). Two of these samples were cloudy, two were clear. In double-diffusion experiments in agarose samples with high levels of HSAP reacted with an antiserum prepared against purified placental AP. The precipitation lines showed AP activity. Extracts from amniotic cells showed no reaction with the above-mentioned antiserum. In previous experiments this antiserum has been shown to react with AP from placenta and intestine but not from liver.

Samples with high enzyme levels were examined by starch gel electrophoresis and subsequent enzyme staining. It was not possible to distinguish HSAP and HLAP electrophoretically. All samples

Table II Heat stable alkaline phosphatase activity in amniotic fluid ($\mu\text{mol } \alpha\text{-naphthol}$ liberated per ml/h) in normal pregnancies and pregnancies complicated by immunization diabetes and toxæmia

Figures within brackets indicate number of samples examined Mean \pm S D

Pregnancy week	Normal pregnancy	Pregnancy complicated by		
		Immunization	Diabetes	Toxæmia
30 and earlier	0.33 \pm 0.29 (47)	0.54 \pm 0.25 (10)	—	—
31-35	0.91 \pm 0.97 (34)	0.72 \pm 0.20 (29)	1.47 \pm 2.97 (13)	0.87 \pm 0.63 (70)
36-40	1.25 \pm 1.06 (74)	0.80 \pm 0.41 (51)	1.05 \pm 1.07 (21)	1.43 \pm 1.53 (25)
41 and later	1.05 \pm 0.71 (74)	—	—	1.69 \pm 0.23 (7)

Table III Acid phosphatase activity in amniotic fluid ($\mu\text{mol } \alpha\text{-naphthol}$ liberated per ml and h) in normal pregnancies and pregnancies complicated by immunization, diabetes and toxæmia

Figures within brackets indicate number of samples examined. Mean \pm S.D.

Pregnancy week	Normal pregnancy	Pregnancy complicated by		
		Immunization	Diabetes	Toxæmia
0 and earlier	0.076 \pm 0.041 (47)	0.109 \pm 0.059 (10)	—	—
1-35	0.073 \pm 0.035 (34)	0.084 \pm 0.053 (79)	0.108 \pm 0.072 (13)	0.087 \pm 0.044 (20)
6-40	0.096 \pm 0.107 (74)	0.077 \pm 0.048 (51)	0.095 \pm 0.056 (21)	0.112 \pm 0.102 (25)
1 and later	0.196 \pm 0.155 (24)	—	—	0.187 \pm 0.064 (2)

if amniotic fluid with high levels of AP showed a wide and rather blurred zone of enzyme activity similar to that found in meconium (Fig. 1). The front of this zone had about the same mobility as AP from fetal intestine. AP in amniotic fluid did not coincide in its electrophoretic mobility with any of the common phenotypes of placental AP. Samples of amniotic fluid with high levels of AcP showed one rather strong and blurred zone of activity (Fig. 1). A zone with the same mobility and appearance was found in meconium. Extracts of placenta and fetal intestine showed different electrophoretic patterns. AP from meconium and fetal intestine was found to be heat stable.

DISCUSSION

The trends as far as acid and alkaline phosphatase levels during normal pregnancy are concerned were in agreement with the findings by Hahnemann & Sørensen (6) and Sutcliffe et al. (13).

This study also agrees with that of Geyer & Schneider (5) who found a lowered level of AP in isoimmunization. We found a decreased level of

HSAP in the period 36th-40th week. Other investigators, however, have not confirmed this (2, 7). No effect of isoimmunization on AcP was found and thus we were not able to confirm the results of Geyer & Schneider (5).

The observation by Roopnanesingh et al. (11) and Fennefrohn (3) of an increased level of HSAP in toxæmia or placental insufficiency is not supported by our study. Their findings might be explained by meconium release especially in cases of fetal distress. Even slight contamination by meconium may give a pronounced increase in the phosphatase level. In our material 11 cases of toxæmia with placental insufficiency showed neither increase nor decrease in the levels of HLAP and HSAP compared with normal pregnancy.

In diabetes mellitus the AcP and AP levels were found to be within normal limits in agreement with previous investigations (8).

Conclusively studies of AcP and AP in amniotic fluid seem to be of little value in the clinical management of complicated pregnancy. This applies to the estimation of the gestational age as well.

It can be questioned whether there may exist

Table IV Acid and alkaline phosphatase activities in samples of amniotic fluid ($\mu\text{mol } \alpha\text{-naphthol}$ liberated per ml and h) contaminated by meconium and blood (Mean \pm S.D.)

	Samples contaminated by	
	Meconium ($n=73$)	Blood ($n=25$)
Heat labile alkaline phosphatase	16.57 \pm 15.15	1.77 \pm 1.79
Heat stable alkaline phosphatase	8.42 \pm 11.9	2.78 \pm 3.47
Acid phosphatase	0.352 \pm 0.343	0.748 \pm 0.614

Table V Relation between acid and alkaline phosphatase activity ($\mu\text{mol } \alpha\text{-naphthol}$ liberated per ml and h) in samples of amniotic fluid contaminated by meconium (Mean \pm S.D.)

	Acid phosphatase activity	
	Below 0.3 μmol per ml and h ($n=15$)	Above 0.3 μmol per ml and h ($n=8$)
Heat labile alkaline phosphatase	13.97 \pm 16.66	21.43 \pm 11.00
Heat stable alkaline phosphatase	1.50 \pm 0.92	21.43 \pm 12.23

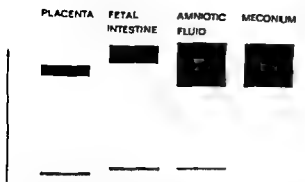


Fig 1 Schematic picture showing the alkaline phosphatase patterns in placenta (S variant), fetal intestine, amniotic fluid contaminated by meconium and meconium after starch gel electrophoresis at pH 8.6. The arrow shows the direction of migration towards the anode.

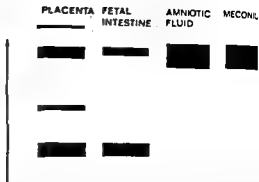


Fig 2 Schematic picture showing the acid phosphatase patterns in placenta, fetal intestine, amniotic fluid contaminated by meconium and meconium after starch gel electrophoresis at pH 8.2. The arrow shows the direction of migration towards the anode.

smaller or rather large contributions of enzymes from meconium without any detectable discolouration of the amniotic fluid. Jonasson (7) and Hahneemann & Sorensen (6) noticed samples of amniotic fluid with high AP levels but without detectable meconium contamination. In this investigation four samples were found in weeks 39–40 with extremely high AP values but without discolouration. If they had been included the mean AP level would have been increased almost threefold. It may be argued that discarding such extreme values is not allowed. But it is well known from amnioscopic or even direct examination of the amniotic fluid that a slight green discolouration is very difficult to detect.

Thus occasional contributions of meconium to samples of amniotic fluid may pass unnoticed and may strongly influence the results. Therefore we think it is correct to discard extreme values of phosphatase which fall outside the normal distribution since it is very plausible in these cases that contamination of meconium has occurred. A technical complication like this will render contradictory results.

Concerning the origin of AcP and AP in amniotic fluid different views have been presented. Sutcliffe & Brock (12) and Roopnarinesingh (11) suggested HSAP to be of placental origin. Changed levels should therefore reflect disturbances in placental function (3, 11). Our results indicate, however, that HSAP in amniotic fluid probably is of intestinal and not of placental origin. Electrophoretically AP in amniotic fluid was different from the common placental AP phenotypes. Furthermore the strong association between the levels of HSAP and AcP in

indicates these enzymes to have a common origin and electrophoretically these enzymes coincided with the patterns found in meconium.

It is difficult to explain why HLAP and HSAP in meconium contaminated samples appear to be associated with the same electrophoretic components. The proportions of HLAP and HSAP vary considerably between samples. One possibility may be that the time between voiding of meconium and sampling may affect the results so that freshly voided meconium contains HSAP and AcP. In time AP may alter its heat stability, e.g. through the action of other enzymes in the fluid, and AcP may gradually lose its activity.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (Project No. 07 2725), the Medical Faculty, University of Umeå and the Perinatal Research Council of Expressen. The skilful technical assistance of Mrs Solveig Berggren is gratefully acknowledged.

REFERENCES

- 1 Beckman L, Beckman G & Magnusson S. Factors influencing the levels of alkaline phosphatases in maternal serum and cord serum. *Hu Hered* 21: 69, 1971.
- 2 Benzie R J, Doran T A, Harkins J L, Jones Owen V M & Porter C J. Composition of amniotic fluid and maternal serum in pregnancy. *A J Obstet Gynecol* 119: 798, 1974.
- 3 Fennefroh B. Fruchtwasseruntersuchungen in den letzten Schwangerschaftswochen. I. Bestimmung von

Bilirubin alkalischer Phosphatase und Cholesterin Z Geburtsh Perinatol 176 233 1972

Fex G Holmberg N G & Löfstrand T Phospholipids and creatinine in amniotic fluid in relation to gestational age during normal pregnancy Acta Obstet Gynecol Scand 54 425 1975

Geyer H & Schneider J Enzyme im Fruchtwasser Z Klin Chem Klin Biochem 8 141 1970

Hahnemann N & Sørensen S A Studies on alkaline phosphatase in amniotic fluid Acta Obstet Gynecol Scand 53 15 1974

Jonasson L E The clinical value of amniotic fluid analysis in pregnancies complicated by Rh immunization or hepatitis Acta Obstet Gynecol Scand 52 113 1973

Kellen J A Kasper D & Leung K K Y Alkaline phosphatase from amniotic fluid Enzym Biol Clin 11 429 1970

Löfstrand T & Fex G The value of amniotic fluid lecithin/sphingomyelin determination in prediction of hyaline membrane disease Acta Obstet Gynecol Scand 55 419 1976

Löfstrand T Fex G & Holmberg N G

Phospholipids and creatinine in amniotic fluid in relation to gestational age II Complicated pregnancy Acta Obstet Gynecol Scand 55 145 1976

11 Roopnanesingh S Morris D & Matadal L Amniotic fluid heat stable alkaline phosphatase in normal pregnancy and in pre eclampsia J Obstet Gynecol Br Comm 79 29 1972

12 Sutcliffe M & Brock D J H Observations on the origin of amniotic fluid enzymes J Obstet Gynecol Br Comm 79 902 1972

13 Sutcliffe R G Brock D J H Robertson J G Scrimgeour J H & Monaghan J M Enzymes in amniotic fluid A study of specific activity patterns during pregnancy J Obstet Gynecol Br Comm 79 895 1972

Submitted for publication April 12 1976

Tord Löfstrand
Department of Obstetrics and Gynecology
Kärnsjukhuset
S-54101 Skövde
Sweden

Tvilling graviditet?

Prognosen för tvillinggraviditet
är sämre än för enfostrig graviditet

Ca 50% av tvillinggraviditeterna
avslöjas först vid förlossningen

Förutsättning för förbättrad prognos
är att diagnosen ställs i god tid

*Screening med hCS (hPL) i 29 e och 30 e graviditetsveckan
innebär att endast ca 10% behöver efterundersökas
med exempelvis ultraljud för att fastställa duplex
Screeninggräns $> 5 \mu\text{g/ml}$ "*

1) Mågeste et al. Läkartidningen 73 (1976) 5 p. 325-326

For screening vid fastställande
av tvillinggraviditet

Phadebas®

hCS (hPL) Test



Pharmacia Norden AB Avd Diagnostika
Box 159 751 04 UPPSALA

 Pharmacia

ORGANIZATION OF AMNIOCENTESIS FOR ANTENATAL GENETIC DIAGNOSIS

Desider J Rothe Lars L Cederqvist Ioannis A Zervoudakis
and Fritz Fuchs

*From the Department of Obstetrics and Gynecology
The New York Hospital Cornell Medical Center New York NY USA*

Abstract In spite of the rapid development of amniocentesis for genetic diagnosis it is still only a small fraction of the mothers at risk who are having the procedure performed. The medical and public health problems associated with genetic amniocentesis are discussed on the basis of the experience gathered in a major medical center.

Prenatal diagnosis of fetal disorders on the basis of examination of amniotic fluid was introduced about 25 years ago when Bevis (1952) examined the content of the bile pigment in cases of erythroblastosis fetalis due to Rh isoimmunization of the mother. Some years later it was demonstrated that examination of the cells in the amniotic fluid could reveal the fetal sex (11 16 24 25) and the fetal ABO blood group (11). Features which both could be used as genetic markers. Rüs & Fuchs (1960) were the first to use antenatal sex determination for the prevention of sex linked hereditary disorders such as hemophilia and one form of muscular dystrophy. Technological advances soon led to attempts to culture amniotic fluid cells for karyotype studies (10) but the first successful reports did not appear until 1966 (26 27). When in the same year it was first predicted that it should be possible to diagnose inborn errors of metabolism before birth by biochemical examination of the amniotic fluid (7) it was not anticipated that ten years later we would be able to diagnose more than 60 different hereditary disorders of this kind. This is just another illustration of the rapid development of the medical sciences and we can take some pleasure from the fact that this happened within the specialty of obstetrics. But although obstetricians can claim some of the credits for the first inroads in this field the subsequent development would not have been pos-

sible without the work of geneticists biochemists and pediatricians. And it would not have been possible without the rapid advances in the technology of tissue culture and the development of new biochemical methods particularly in enzymology during the last two decades. Nor would it have been possible without the detailed characterization of inherited metabolic disorders moving from a study of their clinical manifestations to the demonstration of specific enzyme deficiencies in various tissues and from there to the recognition of such deficiencies in cultures of fibroblasts leukocytes and amniotic fluid cells.

In addition to the inborn errors of metabolism and the chromosomal abnormalities a third group of congenital disorders have been added to those which can be detected before birth the neural tube defects. These disorders are characterized by a high concentration of a specific protein α fetoprotein in the amniotic fluid. Also the level in the maternal plasma is increased permitting screening for these congenital malformations by examination of maternal blood samples in the beginning of the second trimester of pregnancy (4 17 23).

The advantages of antenatal diagnosis of inborn errors of metabolism chromosomal abnormalities and neural tube defects are quite obvious. Many of these disorders lead to severe debilitating disease often with death at an early age. Others are associated with severe mental retardation precluding a normal life. For most of these disorders no treatment exists as yet but if they can be diagnosed early in gestation the birth of afflicted individuals can be prevented by interruption of the pregnancy. However while the prevention is of great advantage both to the individual parents and

Table I Number of genetic amniocenteses performed

Numbers in brackets indicate patients aged 35 and over

Year	At New York Hospital Cornell Medical Center	New York City (estimates)	USA (estimates)
1973	20 (258)	200 (7 000)	1 200 (200 000)
1974	74 (764)	300 (7 000)	3 000 (200 000)
1975	159 (309)	700 (7 000)	5 000 (700 000)

In society at large the development of the necessary public health measures poses considerable problems. Some of these problems are illustrated by our own experience in organizing a program for genetic amniocentesis.

Before 1973 the demand for amniocentesis for genetic diagnosis in our institution was very limited and cases were managed on an individual basis. In 1973 it became evident that a more systematic approach was necessary. A laboratory for culture and karyotyping of amniotic fluid cells was established. In the beginning amniocentesis was recommended only to mothers 40 years of age or older and mothers with balanced translocations or with previous children with chromosomal defects. Gradually the availability of amniocentesis for chromosome studies became known and procedures and guidelines for referral were developed. During the calendar year 1973 only 20 genetic amniocenteses were performed but as Table I illustrates, once the laboratory was firmly established the activity increased rapidly: 74 procedures were done in 1974 and 159 in 1975. The subsequent tables are based on the experience of these two years.

INDICATIONS

When it became evident that the procedure could be carried out with minimal risks even on a fairly large scale we decided to advise all mothers over 37 years of age to have amniocentesis and to perform it even in the age group 35-37 on request in view of the increasing risk of Down's syndrome after 35 (5). As seen in Table II maternal age was by far the most frequent indication. Previous delivery of an infant with Down's syndrome only constituted the indication in 10% of the cases. The maternal age distribution shown in Table III illustrates

that the largest number of requests for amniocentesis came from patients between 38 and 40.

PROCEDURE

Amniocentesis is usually performed at 16-17 weeks of gestation as an out-patient procedure. With a few exceptions all amniocenteses are performed by the investigator (DJR) and we no longer accept amniotic fluid samples collected by obstetricians in their offices unless the patient lives too far away to come to the hospital. Sonography is usually performed before the procedure for placental localization but we do not as a rule perform amniocentesis under sonographic control. Grossly bloody amniotic fluid was obtained in 24% of the taps regardless of whether sonography had been performed or not. The amniotic fluid is placed directly into tissue flasks without centrifugation (6). A sample is taken for determination of a fetoprotein. The elimination of separation of the cells from amniotic fluid has reduced the risk of contamination and increased the growth rate. The average time between amniocentesis and karyotyping is now 18 days.

Rh-negative mothers are given Rh-immunoglobulin at the procedure unless previously immunized or if the husband was known to be Rh-negative. In 1% of the cases amniocentesis had to be repeated because of failure to yield enough cells for karyotyping. All patients refused to have a second amniocentesis and one of these aged 40 subsequently delivered an infant with Down's syndrome.

Genetic counseling is an essential feature of the program and is provided both by the obstetrician, geneticist and a genetic counselor. An informed consent is always obtained at the initial consultation with the patient and her husband. Repeated time-consuming contacts are often necessary particularly during the interval between amniocentesis and diagnosis.

Table II Indications for amniocentesis

Maternal age	197	84.5
Previous trisomy 21	74	10.3
Neural tube defect	8	3.4
Fetal wastage	2	0.9
Sex determination	1	0.4
Previous congenital abnormalities	1	0.4
Total	233	100%

ABNORMAL FINDINGS

The abnormal findings included five cases of Trisomy 21 (Down's syndrome) two cases of Trisomy 18 and one case of mosaic Trisomy F (Table IV). In all instances the pregnancy was interrupted at the request of the mother. All the aborted fetuses were found to be of the predicted sex, a corroboration of the original findings. One patient was 48 years old and the pregnancy was her first after interruption of the pregnancy; she ceased menstruating and adopted a child.

Abnormal chromosomal complement was found in association with normal phenotypes in five cases (Table V). In two cases the fetus carried the same balanced translocation as the phenotypically normal mother. In three cases mosaicism and abnormal cell lines were considered most likely due to artefact or viral contamination. In two cases female infants were predicted from the chromosomal complement but normal male infants were born in both cases. Amniocentesis was performed by obstetricians in their office and inadvertent puncture of the maternal bladder cannot be ruled out. Two errors on a total of 233 examinations gives an accuracy rate of 99%.

COMPLICATIONS

Grossly bloody amniotic fluids were as mentioned obtained in 24% of the cases regardless of whether sonography was performed or not. One major advantage of sonography is illustrated by the following case. A patient requested that amniocentesis be performed without prior sonography to avoid exposure of the fetus to short wave radiation. Amniocentesis was performed without complication and a normal female was predicted. At 38 weeks she delivered normal twins. Sonography would have permitted the early diagnosis of a twin gestation and facilitated the sampling of both amniotic fluids.

A 40-year-old patient aborted one week after a

Table IV Abnormal findings

4 cases of	47,XY +21	Trisomy 21 (Down's syndrome)
1	46,XY +(21 15)	Trisomy 21 (Down's syndrome)
1	47,XY +18	Trisomy 18
1	47,XX +18	Trisomy 18
1	46,XY / 47,XY +F	Mosaic Trisomy F
8 cases—3.4% of total amniocenteses performed		

repeat amniocentesis. Another patient 41 years old aborted 4 weeks after amniocentesis; her pregnancy was complicated by uterine fibroids and hypertension and she had previously had five elective abortions. One patient delivered a stillborn at 35 weeks 12 weeks after a repeat amniocentesis. The first culture showed unusual chromosomal abnormalities compatible with viral infection; the second culture revealed a normal chromosomal complement. No congenital abnormalities were found at autopsy. Thus the total fetal loss was 1.3%.

SEQUELAE

When a fetal abnormality is found and the pregnancy is interrupted it puts the parents under considerable psychological stress, particularly if the couple does not have any previous normal children. Although spared the delivery of a severely affected child the parents have guilt feelings, self reproaches, depressions and marital strains as has been observed by other investigators (3). The effect of such sequelae can be greatly reduced by intensive post-abortion counseling.

DISCUSSION

Our results provide evidence that second trimester amniocentesis is a safe and accurate procedure and they are in complete agreement with the collaborative prospective study of the safety and accuracy of genetic amniocentesis carried out under the direction of the National Institute of Child Health and Human Development. A recent report on that study of 1040 subjects and 992 controls showed a fetal loss of 3.5% for the subjects and 3.2% for the controls and a diagnostic accuracy of 99.4% (19).

The demand for amniocentesis to detect inborn errors of metabolism (8) has been very limited and

Table III Distribution of maternal age

Under 35	79	12.4%
35-37	69	79.3%
38-40	11	37.3%
41-44	45	19.3%
Over 44	3	1.7%
Total	233	100.0%

Table I Number of genetic amniocenteses performed

Numbers in brackets indicate patients aged 35 and over

Year	At New York Hospital Cornell Medical Center	New York City (estimates)	USA (estimates)
1973	20 (258)	700 (7 000)	1 200 (200 000)
1974	74 (764)	300 (7 000)	3 000 (700 000)
1975	159 (309)	700 (7 000)	5 000 (200 000)

to society in large the development of the necessary public health measures poses considerable problems. Some of these problems are illustrated by our own experience in organizing a program for genetic amniocentesis.

Before 1973 the demand for amniocentesis for genetic diagnosis in our institution was very limited and cases were managed on an individual basis. In 1973 it became evident that a more systematic approach was necessary. A laboratory for culture and karyotyping of amniotic fluid cells was established. In the beginning amniocentesis was recommended only to mothers 40 years of age or older and mothers with balanced translocations or with previous children with chromosomal defects. Gradually the availability of amniocentesis for chromosomal studies became known and procedures and guidelines for referral were developed. During the calendar year 1973 only 20 genetic amniocenteses were performed but as Table I illustrates, once the laboratory was firmly established the activity increased rapidly: 74 procedures were done in 1974 and 159 in 1975. The subsequent tables are based on the experience of these two years.

INDICATIONS

When it became evident that the procedure could be carried out with minimal risks even on a fairly large scale we decided to advise all mothers over 37 years of age to have amniocentesis and to perform it even in the age group 35-37 on request in view of the increasing risk of Down's syndrome after 35 (5). As seen in Table II maternal age was by far the most frequent indication. Previous delivery of an infant with Down's syndrome only constituted the indication in 10% of the cases. The maternal age distribution shown in Table III illustrates

that the largest number of requests for amniocentesis came from patients between 38 and 40.

PROCEDURE

Amniocentesis is usually performed at 16-17 weeks of gestation as an out patient procedure. With a few exceptions all amniocenteses are performed by the investigator (DJR) and we no longer accept amniotic fluid samples collected by obstetricians at their offices unless the patient lives too far away to come to the hospital. Sonography is usually performed before the procedure for placental localization but we do not as a rule perform amniocentesis under sonographic control. Grossly bloody amniotic fluid was obtained in 24% of the cases regardless of whether sonography had been performed or not. The amniotic fluid is placed directly into tissue flasks without centrifugation (6). A sample is taken for determination of a fetoprotein. The elimination of separation of the cells from amniotic fluid has reduced the risk of contamination and increased the growth rate. The average time between amniocentesis and karyotyping is now 18 days.

Rh negative mothers are given Rh-ogam at the procedure unless previously immunized or if the husband was known to be Rh negative too. In 11 of the cases amniocentesis had to be repeated because of failure to yield enough cells for karyotyping. All patients refused to have a second amniocentesis and one of these aged 40 subsequently delivered an infant with Down's syndrome.

Genetic counseling is an essential feature of the program and is provided both by the obstetrician, geneticist and a genetic counselor. An informed consent is always obtained at the initial counseling session with the patient and her husband but repeated time consuming contacts are often necessary particularly during the interval between amniocentesis and diagnosis.

Table II Indications for amniocentesis

Maternal age	197	84.5%
Previous trisomy 21	74	10.3%
Neural tube defect	8	3.4%
Fetal wastage	2	0.9%
Sex determination	1	0.4%
Previous congenital abnormalities	1	0.4%
Total	233	

ABNORMAL FINDINGS

he abnormal findings included five cases of Trisomy 21 (Down's syndrome) two cases of Trisomy 18 and one case of mosaic Trisomy F (Table V). In all instances the pregnancy was interrupted at the request of the mother. All the aborted fetuses were found to be of the predicted sex, a corroboration of the original findings. One patient was 48 years old and the pregnancy was her first after interruption of the pregnancy; she ceased menstruating and adopted a child.

Abnormal chromosomal complement was found in association with normal phenotypes in five cases (Table V). In two cases the fetus carried the same balanced translocation as the phenotypically normal mother. In three cases mosaicism and abnormal cell lines were considered most likely due to artefact or viral contamination. In two cases male infants were predicted from the chromosomal complement but normal male infants were born in both cases; amniocentesis was performed by obstetricians in their office and inadvertent rupture of the maternal bladder cannot be ruled out. Two errors on a total of 233 examinations gives an accuracy rate of 99%.

COMPLICATIONS

Grossly bloody amniotic fluids were as mentioned obtained in 24% of the cases regardless of whether sonography was performed or not. One major advantage of sonography is illustrated by the following case. A patient requested that amniocentesis be performed without prior sonography to avoid exposure of the fetus to short wave radiation. Amniocentesis was performed without complication and a normal female was predicted. At 38 weeks she delivered normal twins. Sonography would have permitted the early diagnosis of a twin gestation and facilitated the sampling of both amniotic fluids.

A 40-year-old patient aborted one week after a

Table IV Abnormal findings

4 cases of	47 XY +21	Trisomy 21 (Down's syndrome)
1	46 XY +(21 15)	Trisomy 21 (Down's syndrome)
1	47,XY +18	Trisomy 18
1	47,XX +18	Trisomy 18
1	46,XY / 47,XY +F	Mosaic Trisomy F
8 cases—3.4% of total amniocenteses performed		

repeat amniocentesis. Another patient 41 years old aborted 4 weeks after amniocentesis; her pregnancy was complicated by uterine fibroids and hypertension and she had previously had five elective abortions. One patient delivered a stillborn at 35 weeks 12 weeks after a repeat amniocentesis. The first culture showed unusual chromosomal abnormalities compatible with viral infection; the second culture revealed a normal chromosomal complement. No congenital abnormalities were found at autopsy. Thus the total fetal loss was 1.3%.

SEQUELAE

When a fetal abnormality is found and the pregnancy is interrupted it puts the parents under considerable psychological stress, particularly if the couple does not have any previous normal children. Although spared the delivery of a severely affected child the parents have guilt feelings, self reproaches, depressions and marital strains as has been observed by other investigators. (3) The effect of such sequelae can be greatly reduced by intensive post abortion counseling.

DISCUSSION

Our results provide evidence that second trimester amniocentesis is a safe and accurate procedure and they are in complete agreement with the collaborative prospective study of the safety and accuracy of genetic amniocentesis carried out under the direction of the National Institute of Child Health and Human Development. A recent report on that study of 1040 subjects and 992 controls showed a fetal loss of 3.5% for the subjects and 3.2% for the controls and a diagnostic accuracy of 99.4% (19).

The demand for amniocentesis to detect errors of metabolism (8) has

Table III Distribution of maternal age

Under 35	29	12.4%
35-37	69	29.3%
38-40	87	37.3%
41-44	11	19.3%
Over 44	3	1.7%
Total	233	100.0%

Table V *Abnormal karyotypes with normal phenotypes*

Karyotype	Phenotype	Comment
46,XX 5p- 22q+	Normal female with balanced translocation	Mother: same translocation
46,XX 1q+ 8q-	Normal female with balanced translocation	Mother: same translocation
46,XX / 47,XX+13 11 cells / 1 cell	Normal female	? Contamination
1 46,XY / aneuploidy ranging from 45-47 11 cells / 4 cells	Normal male	
2 46,XY 13 cells	Stillborn male at 37 weeks no congenital abnormalities	? Viral amnionitis
46,XX / 47,XX+E / 46,XX Dq-Cp+ 47 cells / 1 cell / 2 cells	Normal female	? Artefact

not justified the development of a laboratory with the necessary biochemical capabilities. We have managed such cases either by referral of the patients to appropriate centers or by sending amniotic fluid samples to laboratories with documented expertise in regard to the disorders in question. A more pressing need has been to develop a program for antenatal detection of neural tube defects by determination of α fetoprotein in amniotic fluid and maternal blood: the incidence of these congenital malformations is almost the same order of magnitude as the incidence of all the detectable genetic disorders together.

in developing facilities and experience for antenatal detection of genetic disorders is to that of others (1, 12, 13, 15, 18, 20). Once the procedures and lines of referral have been established, the demand increases rapidly. Even so far from all the patients at risk are getting amniocentesis. As seen in Table I there were 309 patients aged 35 or over who gave birth in our institution during 1975, but we only performed 159 amniocenteses. Not all the patients were seen early enough in pregnancy to have genetic amniocentesis; on the other hand, some of our amniocenteses were performed on patients who delivered elsewhere. For the City of New York, the situation was worse with only about 10% of the estimated number at risk having amniocentesis. For the country as a whole, the estimated figure for 1975 is only 2.5%. Although a 1974 survey by Kaback & Levine demonstrated a rapid growth both in the number of centers performing genetic amniocentesis and the number of amniocenteses performed by each

center, there is a long way to go before all the patients at risk have access to the procedure.

To cover the population at risk will thus require the development of a very large number of facilities. Is it worth the effort? A group of concerned scientists in New York has made a calculation of the costs for a program for the City (22). They estimated that about 8000 amniocenteses would have to be carried out annually to detect and prevent 60 cases of Down's syndrome. At \$700 per amniocentesis and excluding start-up costs, the annual expenditure would be \$1 600 000, as compared to an estimated cost for the lifelong care of 60 cases of Down's syndrome of \$12 000 000.

Previous studies and our own experience all provide strong support for the view that amniocentesis for genetic diagnosis should be organized on a regional basis. Expertise in karyotyping and genetic counseling is scarce and can be best utilized if located in tertiary centers of obstetric care. Where necessary, however, amniotic fluid samples can be shipped over long distances, even from one continent to another, without the cells losing their potential for growth in culture (11a).

REFERENCES

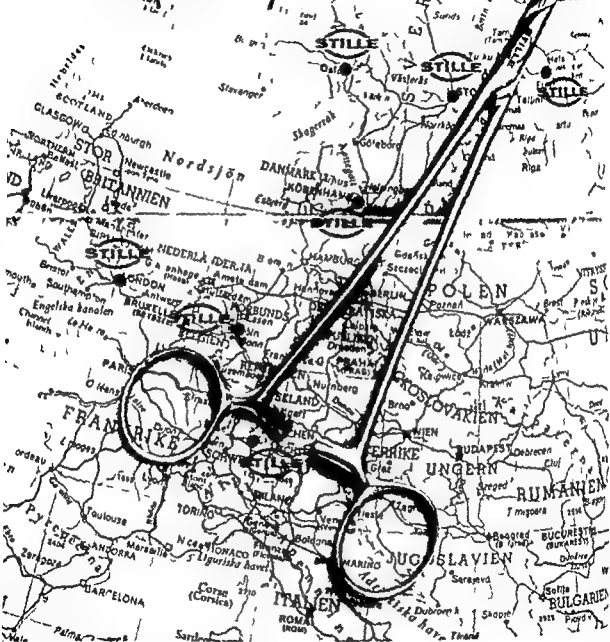
- 1 Bartsch F K, Lundberg J & Wahlström J. The technique, results and risks of amniocentesis for genetic reasons. *J Obstet Gynaecol Br Comm* 82: 991, 1974.
- 2 Bevis D C A. The antenatal prediction of haemolytic disease of the newborn. *Lancet* i: 395, 1972.
- 3 Blumberg B D, Golbus M S & Hanson K E. The psychological sequelae of abortion.

- a genetic indication *Am J Obstet Gynecol* 127 799 1975
- 1 Brock D J H & Sutcliffe R G Alpha fetoprotein in the antenatal diagnosis of anencephaly and spina bifida *Lancet* 2 197 1972
- 2 Carter C O & Evans A A Risk of parents who have had one child with Down's syndrome (mongolism) having another child similarly affected *Lancet* 2 785 1961
- 3 Cederqvist L L Wennerstrom C Senterfit L D Baldrige P H & Rothe D J Simplified method for accelerated growth of amniotic fluid cell cultures *Am J Obstet Gynecol* 116 871 1973
- 4 Fuchs F Genetic information from amniotic fluid constituents *Clin Obstet Gynecol* 9 565 1966
- 5 Fuchs F Prenatal diagnosis of inborn errors of metabolism *In* Recent Progress in Obstetrics and Gynecology (ed L S Persanianov T V Chervakova and J Presl) pp 199-212 Excerpta Med Amsterdam 1974
- 6 Fuchs F & Cederqvist L L Use of amniotic fluid cells in prenatal diagnosis *In* Amniotic Fluid (ed D V I Fairweather and T K A B Eskes) Excerpta Med Amsterdam 1st ed 1973 2nd ed in print
- 7 Fuchs F Freeseleben E Knudsen E E & Rus P Determination of foetal blood group *Lancet* 1 996 1956
- 8 Fuchs F & Philip J Mulighed for antenatal undersogelse af fostrets kromosomer (Possibility of antenatal examination of fetal chromosomes) *Proc Dan Soc Obstet Gynecol* 1961-62 ■ 42 *Nord Med* 69 572 1963
- 9 Fuchs F & Rus P Antenatal sex determination *Nature* 177 330 1956
- 10 Gadow E C Paz J S Castilla E E Rothe D J & Cederqvist L L Prenatal detection of chromosome aberrations after intercontinental transport of amniotic fluid *Int J Gynaecol Obstet* 14 165 1976
- 11 Jacobson C G & Barter R B Intrauterine diagnosis and management of genetic defects *Am J Obstet Gynecol* 99 796 1967
- 12 Laxova R Lewis B V & Suddaby M A clinical service for prenatal diagnosis *Lancet* 2 964 1975
- 13 Levine M H Kaback M M & Griffith C Prenatal genetic diagnosis in North America: Abstr Annual Meet Am Soc Hum Gen 1975
- 14 Lindsten J Therkelsen A J Friedrich U Jonasson J Steenstrup O R & Wiquist N Prenatal cytogenetic diagnosis *Int J Gynaecol Obstet* 12 101 1974
- 15 Makowski E L Prem K A & Kaiser I H Detection of sex of fetuses by the incidence of sex chromatin body in nuclei of cells in amniotic fluid *Science* 123 542 1956
- 16 Milunsky A & Alpert E Prenatal diagnosis of neural tube defects *Obstet Gynecol* 48 1 and 6 1976
- 17 Nadler H L & Gerbie A B Role of amniocentesis in the intrauterine detection of genetic disorders *New Engl J Med* 282 596 1970
- 18 National Institute of Child Health and Human Development Study Group Midtrimester amniocentesis for prenatal diagnosis: safety and accuracy *JAMA* 236 1471 1976
- 19 Philip J Bang J Hahnemann N Mikkelsen M Niebuhr H Rebbe H & Weber J Chromosome analysis of fetuses in risk pregnancies *Acta Obstet Gynecol Scand* 53 9 1974
- 20 Rus P & Fuchs F Antenatal determination of foetal sex in prevention of hereditary diseases *Lancet* 2 180 1960
- 21 Scientists Committee for Public Information Inc Taskforce on Genetic Amniocentesis Personal communication 1975
- 22 Seppala M & Ruoslahti H Alpha fetoprotein physiology and pathology during pregnancy and application to antenatal diagnosis *J Perinat Med* 1 104 1973
- 23 Serr D M Sachs L & Danon M Diagnosis of sex before birth using cells from the amniotic fluid *Bull Res Council Israel* 5B 137 1955
- 24 Shettles L B Nuclear morphology of cells in human amniotic fluid in relation to sex of infant *Am J Obstet Gynecol* 71 834 1956
- 25 Steele M W & Breg W H Chromosome analysis of human amniotic fluid cells *Lancet* 1 383 1966
- 26 Thiede H A Creasman W T & Metcalfe S Antenatal analysis of the human chromosomes *Am J Obstet Gynecol* 94 589 1966

Submitted for publication Nov 22 1976

Fritz Fuchs
Department of Obstetrics and Gynecology
The New York Hospital Cornell Medical Center
525 East 68th Street
New York NY 10021
USA

There is no substitute for quality



AB STILLE-WERNER

Box 43051, S 100 72 STOCKHOLM SWEDEN

A/S Stille Werner C F Riche vej 103 DK 2000 København DANMARK

OY Stille AB Arkadiankatu 12 A SF-00100 Helsinki FINLAND

Stille A ■ Postboks 61 Leirdal Oslo 10 NORGE

Stille AG Postfach CH-8038 Zurich SCHWEIZ

Stille Werner (UK) Ltd 24 York Road Maidenhead Berkshire SL6 1SF ENGLAND

Stille GmbH Zulpicher Platz 7 D 5000 Köln 1 BRD

MANUFACTURING AND SALE OF surgical instruments operating
tables medical technical instruments disposable specialties

STILLE

10 year
guarantee

DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS OF INTRAPARTUM FETAL pH MEASUREMENT

Carl Wood

From the Department of Obstetrics and Gynaecology Monash University
The Queen Victoria Memorial Hospital Melbourne Victoria Australia

Abstract (1) Measurement of fetal scalp blood pH aids the detection of fetal asphyxia. The investigation is done on infants whose fetus is at increased risk of death. (2) Special care in the collection and measurement of scalp blood pH is necessary to obtain satisfactory results. A change of 0.05 pH unit is significant. (3) Acidosis is present when scalp pH is <7.25. (4) A severe acidosis pH 7.10 indicates a worse fetal prognosis. (5) Fetal metabolic acidosis is the commonest type of acidosis but is less hazardous than asphyxial acidosis. (6) Fetal metabolic acidosis is associated with use of drugs. (7) Fetal growth retardation, amniotomy and maternal starvation. (8) The possibility of transient acidosis needs to be taken into account when interpreting fetal scalp pH measurements. (9) Fetal scalp blood studies have helped in finding the influence upon fetal condition of maternal hyperoxygenation, duration of birth, maternal posture and the influence of drugs on fetal condition. (10) A controlled trial has shown that fetal intensive care, routine FHR monitoring and selected fetal scalp blood pH measurement in a high risk group is associated with improved biochemical and neurological status of the newborn. (11) Over the last eight years there has been a significant reduction of intrapartum anoxic stillbirths at the Queen Victoria Hospital. One of the probable reasons for this reduction in intrapartum stillbirths is the introduction of fetal diagnostic techniques. (12) Fetal heart rate monitoring is more help to the clinical obstetrician than scalp sampling—it is applicable both antepartum and intrapartum, more easily organized and more easily learnt.

Methodology

Scalp sampling is mainly used as a means to estimate fetal blood pH. The normal range is two standard deviations of scalp blood pH is 7.25-7.45 (7).

The confidence limits of scalp blood pH measurement 0.05 pH units is twice as large as cord blood (7). The variation of fetal scalp pH is mostly due to the capillary blood varying between arterial and venous blood in its composition. Be-

cause of the greater variation of measurement change of scalp pH can only be considered relevant when it is more than 0.05 pH unit.

For a variety of reasons, mean values from different centres differ significantly (6). The mean values from fourteen centres fall into three distinct statistical categories ranging from 7.29 to 7.38 (Table I). Therefore it is important for each hospital to determine their own normal mean and range. In our laboratory acidosis has been defined as <7.25. To establish this normal mean we used two criteria: population statistics which defined an abnormal population when pH was <7.25 and the Apgar score which also tended to decline when pH was <7.25 (6).

Table I Variability of pH in different centres

Mean pH	Studies
7.29-7.30	6
7.32	2
7.35-7.38	6

Table II Relationship between Apgar score and fetal scalp blood pH

pH	No. of fetuses with Apgar score		Incidence of Apgar 0-3 (%)
	0-3	4-10	
≥7.25	47	298	17
<7.25	27	46	37

$$\chi^2=26 \quad P<0.001$$

Table III Apgar score and severity of acidosis

	Apgar score		Incidence apgar 0-3
	0-3	4-10	
pH < 7.10	12	2	86%
pH 7.10-0.24	15	31	33%

$$\chi^2=10.5 \quad P<0.001$$

Table IV Type of acidosis

	pH	pO ₂	O ₂ sat %	Apgar score	n
Asphyxial	7.10	13	24	4.1	18
Metabolic	7.18	24	43	6.8	20
Normal	7.35	23	53	9.5	12

Table V Narcotics

	Maternal pH change	Fetal pH change
Heroin	-0.04	-0.04
Nalorphine	-0.03	-0.03
Morphine	-0.03	-0.03
Pethidine	-0.02	-0.02

Table VI

	Normal	Hypertension	Hypertension and proteinuria
n	21	151	111
pH	7.35	7.31	7.28
PCO ₂	47	47.5	49
PO ₂	23	22	23
BE _p	-0.5	-1.8	-2.9

Table VII Fetal scalp blood measurements in the normal and growth retarded fetus

Growth retardation	pH	PCO ₂ mmHg	PO ₂ mmHg	BE _p mEq
None	7.35	46.8	23.4	-0.3
n	21	12	12	12
Moderate (3rd-10th percentile)	7.289	45.6	22.1	-3.0
n	31	13	14	14
Severe (<3rd percentile)	7.223	54.5	20.9	-3.4*
n	13	8	8	8

Mann Whitney U test $p<0.001$

NORMAL ESTROL

LOW ESTROL

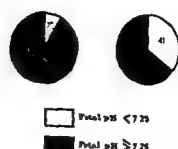


Fig. 1 Percentage of patients with pH < 7.25 in an ($n=56$) and low estrol ($n=71$) groups. The difference between the groups was significant $p<0.001$.

Acidosis and Apgar score

When acidosis is present pH is < 7.25 then three babies are born with an Apgar (Table II) (8). Not only is the presence of acid important but so is the severity of acidosis important (Table III) (8). When pH is < 7.10, two in three babies have an Apgar score of 0-3 in this situation immediate delivery is indicated.

Type of acidosis

Classification of the type of acidosis is also important but this information has not been generally applied clinically because of the difficulty in getting reliable measurement of low PO₂ levels in laboratories.

Asphyxial acidosis is defined when PO₂ is < 6 mmHg and PCO₂ is > 60 mmHg (9). Asphyxial acidosis is usually severe and is often associated with a fetus born in poor condition (Table IV). The various obstetric causes of asphyxia such as abruptio placenta and cord complication are well known.

Metabolic acidosis is usually mild and the fetus is usually born in good condition (Table IV). The

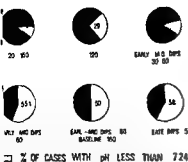


Fig. 2 FHR in relation to fetal scalp pH. The percentage of cases with pH < 7.25 is shown for each FHR group. FHRs are synonymous with decelerations. The incidence of fetal acidosis in the last two groups are significantly different from the normal FHR group (11).

are various causes of metabolic acidosis. Controlled trials using placebo and narcotic injections have shown that single standard dose injections of narcotic agents produce a mild metabolic acidosis in the fetus (Table V) (1). Heroin has a more marked effect than demerol. pH changes more markedly than shown when large or repeated doses of narcotics are given at short intervals.

Fetal metabolic acidosis also occurs in hypertension either with or without proteinuria (Table VI). His metabolic acidosis may be due to the disease process itself, the use of large and frequent doses of narcotic agents in this condition or possibly the use of other drugs. Asphyxia, the commonly held mechanism thought to influence the fetus in this disease, is much less frequent and is only seen when the fetus is born in poor condition. Other causes of metabolic acidosis are fetal growth retardation (Table VII) (12), amniotomy and maternal starvation (1). Also the latter two, amniotomy and maternal starvation, are associated with maternal metabolic acidosis.

Acidosis may be temporary and when this oc-

Table VIII Apgar score and temporary acidosis

	Apgar score	
	0-3	4-10
H < 7.0		
temporary	0	15
persistent	15	31

$\chi^2 = 5.91$ $P < 0.025$

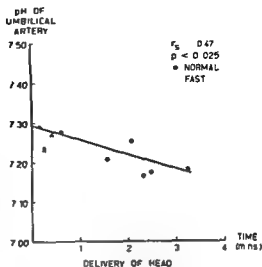


Fig. 3 The pH of the umbilical artery was significantly lower with prolongation of the time taken for delivery of the head (10).

curs does not markedly influence Apgar score (8) (Table VIII). A small series of proven cases of temporary acidosis where serial pH measurements were made had no very low Apgar scores. The importance of temporary acidosis lies in the interpretation of mild to moderate degrees of acidosis in clinical management. A pH between 7.15-7.25 may be wholly or partly due to temporary causes. If pH is < 7.15 a temporary cause is unlikely. Temporary acidosis may occur at the time of amniotomy in the supine hypotensive syndrome following the use of narcotics or in association with transient bradycardia of the fetus perhaps resulting from cord compression.

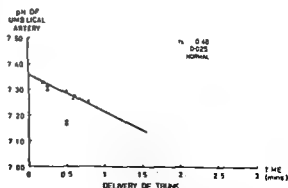


Fig. 4 The pH of the umbilical artery was significantly lower with prolongation of the time taken for delivery of the trunk and limbs (10).

CONTROLLED TRIAL - SPEEDING BIRTH

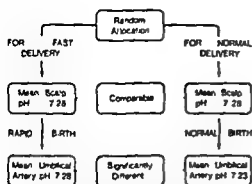


Fig 5 Diagrammatic scheme of controlled trial testing the effects of speeding birth. Thirty patients were in the trial. The two groups were matched for obstetric variables. The umbilical artery blood pH in the fast group was significantly higher than in the normal group and that reported in six of seven studies of normal deliveries in other hospitals (10).

Acidosis and other fetal diagnostic tests

As might be predicted acidosis is found more commonly when oestriol measurement is low (Fig 1) (2). When oestriol excretion is less than the tenth percentile on two consecutive occasions acidosis is subsequently found in 40% of cases whereas acidosis only occurs in 7% of cases when oestriol is normal. In many instances oestriol production and excretion may be reduced without influencing fetal base status. The combination of low oestriol

CONTROLLED TRIAL - POSTURE IN SECOND STAGE

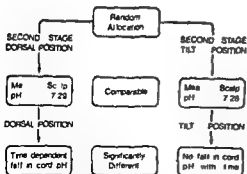


Fig 6 Diagrammatic scheme of controlled trial testing the effects of the dorsal and left tilt positions upon the fetus during the second stage. Fall in pH (scalp capillary umbilical artery pH difference) was significant in dorsal but not left tilt position. Dorsal $r=0.68$, $p<0.01$, $n=20$. Left tilt $r=0.11$, p ns, $n=20$.

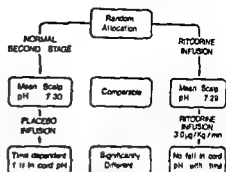
CONTROLLED TRIAL - β MIMETIC INFUSION IN SECOND STAGE

Fig 7 Diagrammatic scheme of controlled trial testing the effects of nitroglycerine (β mimetic) infusion in the second stage. Comparison of regression lines between ($n=10$) and nitroglycerine infusion ($n=15$) groups significant differences for umbilical venous pH, PCO_2 , and umbilical arterial PO_2 and PCO_2 . It abolished the fetal asphyxia which normally occurred in the late second stage (3).

production and acidosis at the time of induction of labour usually warrants Caesarean section in experienced hands.

It is well known that abnormal FHR relates to acidosis and in our studies the most strong correlation is found between late deceleration and acidosis approximately 60% (Fig 2) (11). By using computerized statistical analysis Hon and his colleagues (5) have shown a correlation between certain FHR changes and acidosis in 70–80% of patients. However, one would not expect an absolute correlation between pH and FHR abnormalities for a variety of reasons.

Table IX Neonates

	Control	Intensive care
Apgar score		
0–3	6	4
4–6	19	24
7–10	150	147
Resuscitation at birth		
Nil	5	6
Routine	115	131
Intensive	35	37
Neonatal intensive care	30	11
Neurological signs and symptoms	13	2

* $\chi^2=8.95$, $p<0.001$

$\chi^2=10.03$, $p<0.001$

Fetal Intensive Care Trial

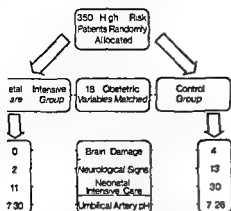


Fig 8 Diagrammatic scheme of controlled trial testing the influence of fetal intensive care upon the high risk fetus. The intensive care ($n=175$) and control ($n=175$) groups were matched for 18 obstetric variables. Significant differences were found in the neurological and biochemical status of the newborn and the number requiring neonatal intensive care. The four babies in the control group had severe brain damage which was still present six months after birth.

In clinical practice we use both FHR and scalp blood pH. FHR is used routinely in high risk cases. Scalp blood pH is done only when FHR abnormality is present and no potentially reversible cause of FHR abnormality is evident.

Birth asphyxia

Scalp pH has helped in assessing other factors which may influence the fetus in labour. Of practical importance to the obstetrician is an understanding of the asphyxia associated with birth. The pH and PCO_2 of cord blood as related to the time taken for delivery of the head (Fig 3) and the time taken for delivery of the trunk (Fig 4) (10). Time limits for these phases of birth have been suggested for our hospital. This is particularly important in a teaching hospital where delay at birth might occur because obstetric attendants are inexperienced. The basis of the time limit was made from examining regression lines and estimating the times over which pH shifts 1 unit. The head delivery time should not exceed 40 minutes and the trunk delivery time 45 seconds.

The birth acidosis and asphyxia may be decreased by speeding delivery (Fig 5) (10) avoiding prolonged use of the dorsal position in the second

Table X Neonatal diagnoses

Diagnoses	Control	Intensive care
Brain damage	4	—
Perinatal hypoxia	3	1
Cerebral hypoxia/irritation	2	—
Apnea/cyanotic attacks	2	—
Tachypnea	2	—
Uncertain	2	1
Hypoglycemia	1	—

stage (Fig 6) (4) or by the use of nitroglycerine (Fig 7) (3).

There is a view that mild asphyxia may be a physiological change associated with birth which is important in the adaptation of the fetus to extra uterine life. However we found no evidence to support this hypothesis as the fetus suffering the mild asphyxia of birth showed no evidence of clinical benefit over those suffering none.

An excess of birth asphyxia should be avoided by placing time limits on the birth process. If the fetus is thought already to be at risk the extent of birth asphyxia may be reduced by expediting delivery.

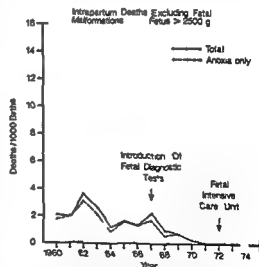


Fig 9 The reduction of intrapartum anoxic stillbirths over ten years at the Queen Victoria Hospital. $r=0.89$, $p<0.001$. Comparison of intrapartum deaths before and after 1967 showed a significant difference $\chi^2=25.39$, $p<0.001$. Although a number of factors are involved in the decline in intrapartum stillbirth rate, the introduction of fetal diagnostic tests is thought also to have contributed to this change.

and avoiding prolonged positioning of the patient in the dorsal position

Effect of monitoring on fetal condition

The use of scalp sampling for routine fetal monitoring has been associated with a lower perinatal mortality in several centres. Used in conjunction with FHR monitoring in a carefully controlled trial in our own institute demonstrated that monitoring and scalp sampling was associated with improved neurological and biochemical status of the newborn and less need for neonatal intensive care (Fig 8) (Tables IX-X). (This trial will be published in more detail subsequently.) The two groups were matched for 18 obstetric variables. The improved status of the newborn in the intensive care group was thought to be due to the more accurate diagnosis of fetal distress rather than other factors involved in the trial. Caesarean section and forceps delivery were not more common in the intensive care group.

The use of FHR monitoring and scalp sampling may have contributed to the decline in intrapartum anoxic stillbirth in our own institution (Fig 9). Fetuses >2500 g no longer die in labour.

ACKNOWLEDGEMENTS

I wish to acknowledge the help of the Obstetric and Nursing Staff of the Queen Victoria Hospital and its members of the University Department who have helped in these studies.

REFERENCES

- 1 Chang A. The effects of some drugs on fetal acid base status. Ph.D. Thesis Monash University Melbourne 1976.

- 2 Flegner J H, Renou P, Wood C, Beischer V A & Brown J B. Correlation between urinary excretion and fetal acidosis in high-risk pregnancy. *Am J Obstet Gynecol* 105: 257 1969.
- 3 Humphrey M, Chang A, Gilbert M & Wood C. The effect of intravenous nitroglycerine on the acid-base status of the fetus during the second stage of labour. *Obstet Gynaecol Br Comm* 87: 734 1975.
- 4 Humphrey M, Chang A, Wood E C, Morgan C & Hounslow D. A decrease in fetal pH during the second stage of labour when conducted in the d.y.s. position. *J Obstet Gynaecol Br Comm* 81: 600 1974.
- 5 Lowensohn M C, Yeh S Y, Forsythe A & Ha E H. Computer assessed fetal heart rate pattern and fetal scalp pH. *Obstet Gynecol* 190: 1974.
- 6 Lumley J, McInnon L & Wood C. Lack of agreement on normal values for fetal scalp blood. *Obstet Gynaecol Br Comm* 78: 13 1971.
- 7 Lumley J, Porter M, Newman, W, Talbot, J, McK, Wakefield E & Wood C. The unreliability of a single estimation of fetal scalp blood pH. *J Lab Clin Med* 93: 5 1971.
- 8 Lumley J & Wood C. Fetal acidosis. *Aust N Z J Obstet Gynaecol* 9: 145 1969.
- 9 Lumley J & Wood C. Unexpected oxygen tension in fetal acidosis. *J Perinatal Med* 1: 66 1973.
- 10 Wood C, NG K, H Hounslow D & Benning H. Time—an important variable in normal delivery. *Obstet Gynaecol Br Comm* 80: 95 1973.
- 11 Renou R & Wood C. Interpretation of the continuous fetal heart rate record. *In Clinics in Obstetrics & Gynaecology Fetal Medicine* vol 1 no 1 (ed R A Beard) W B Saunders Co 1974.
- 12 Wood C. Symposium on Small for Dates Infants. Feto-Placental Physiology. Canberra 1971.

Submitted for publication Feb 27 1977

Carl Wood
Department of Obstetrics and Gynaecology
Monash University
Queen Victoria Memorial Hospital
Melbourne Victoria
Australia

PERINATAL MORTALITY IN HYPERTENSIVE PREGNANT PATIENTS ITS REDUCTION IN A DEVELOPING COUNTRY

Bussamara Neme and Ivo Behle

From the Obstetric Clinic of the Sao Paulo University Medical School
Sao Paulo, Brazil

ious authors (2 28 48) have called our attention to the great disparity existing in the curves of maternal and perinatal mortality. They have pointed out that the latter has decreased relatively little since 1945.

Among other reasons, this was due to limited interest in studying the physiology of the human fetus (1). This has changed within the last ten years, however. Because of studies of amniotic fluid, to maternal hormones and the application of embryology, biochemistry, biophysics and electronics, the study of biological phenomena, knowledge about the fetus has been considerably increased.

Hypertension is one of the clinical conditions which concerns most obstetricians. It stands out because of its high incidence (8 11 18 25 47) and various fetal prognosis (2 7 9 12 15 22 23 25 32 46). Its manifestation and intensity are particularly important in developing countries, where prenatal assistance and methods for assessing fetal well-being and maturity are limited.

Considering these special conditions of developing nations (within which we are included), we decided to see how far simple tests easily applied and interpreted could reduce fetal loss. These tests could be used to calculate the timing of opportune therapeutic preterm delivery in hypertensive pregnant patients.

MATERIAL AND METHODS

The study consisted of 596 hypertensive pregnant patients hospitalized in the Obstetric Clinic of Sao Paulo University Medical School and in the Casa Maternal e da Infancia.

All the patients were in the third trimester of pregnancy and their arterial pressure was always higher than 14x9

mmHg. They were all under the care of a team of doctors charged especially with the treatment of the hypertensive pregnant patients. This consisted fundamentally of hospitalized in the Obstetric Clinic of Sao Paulo University lateral decubitus, low sodium, low carbohydrate, low fat, high protein diet, plenty of water, sedatives, hypotensive drugs (when the pressure was over 18x11 mmHg), psychotherapy and diuretics.

These 596 patients were divided into 4 groups according to the methods employed in order to monitor the well-being and maturity of the fetus.

Group I (1962-1967): 214 patients in which fetal welfare and maturity were evaluated clinically only: fetal heart rate, active fetal movements, cessation of uterine growth, date of last menstruation, height and circumference of the uterus, reduction in volume of the amniotic fluid, etc.

Group II (1968-1973): 181 pregnant patients in which fetal well-being was monitored by amniocentesis and amniocentesis (signs of meconium). Maturity was estimated by creatinine analysis, cytology (orange cells) of the amniotic fluid and by Clements test (estimation of fetal pulmonary surfactants).

Group III (1973): 90 pregnant patients in which in addition to the tests mentioned in Group II, fetal well-being was also evaluated by exercise (4) and tolerance to oxytocin (10-30) tests using electronic equipment.

Group IV (1974-1975): 111 pregnant patients in which fetal well-being and maturity were evaluated by the same tests used in Group III, except that in place of electronic monitoring for the stress tests we used only simple clinical

Table I Perinatal mortality (1975)

Groups	Mortality		
	Stillbirth	Neo-natal	Perinatal
	No	No	No
I 214 cases (1962-67)	23	107	16 39
II 181 cases (1968-73)	5	27	9 14
III 90 cases (1973)	0	0	1 1
IV 111 cases (1974-75)	7	18	2 4
			18 36

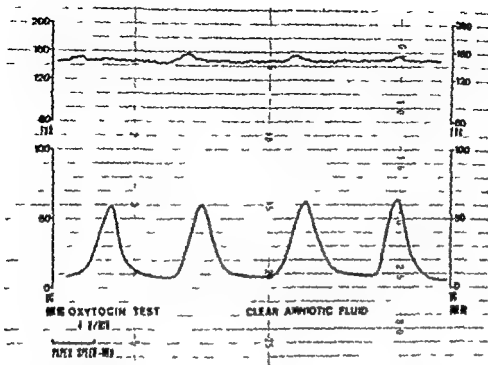


Fig 1 Negative oxytocin test

methods. In these cases fetal heart rate was monitored by Dopstone and uterine activity by manual palpation.

Tests of fetal health were performed from the twenty eighth week. Whenever possible therapeutic preterm delivery was postponed to the thirty fourth week or if possible to the thirty sixth week of pregnancy.

A Saling (36) cannula and a Rodrigues Lima (33) acrylic amnioscope were used for the amnioscope. Amniocentesis was always trans abdominal and umbilical and supra pubic. The presence of meconium in the amniotic fluid was always considered to be a sign of fetal distress justifying immediate (meconium

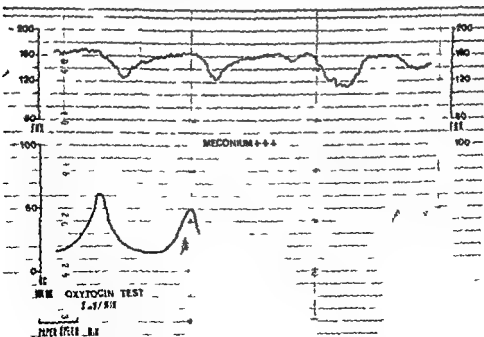


Fig 2 Positive oxytocin test

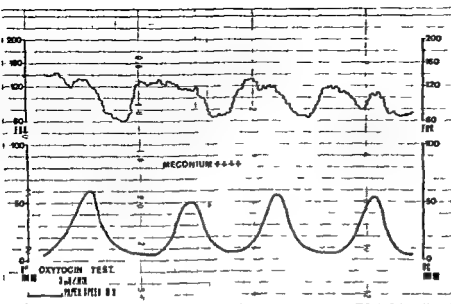


Fig 3 Positive oxytocin test

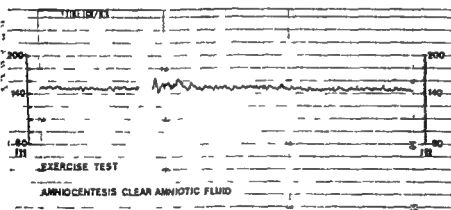


Fig 4 Negative exercise test.

Table II Perinatal mortality and newborn weights

groups	Newborn weights						Perinatal mortality (%)
	-1 500 g		1 501-2 500 g		2 501 g		
	No	%	No	%	No	%	
I (276 newborn)	14	69	61	30.5	143	63.3	18.2
I (184 newborn)	5	5	2.7	28.3	125	67.9	7.7
I (91 newborn)	5	50	5.5	54.9	36	39.5	1.1
(111 newborn)	4	27	3.6	24.3	80	77.0	3.6

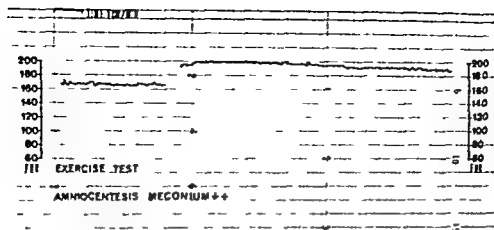


Fig 5 Positive (tachycardia) exercise test.

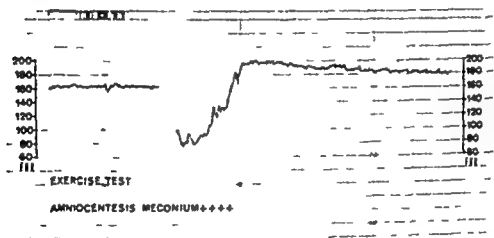


Fig 6 Positive (bradycardia) exercise test

*) or slightly delayed (meconium and) preterm therapeutic delivery

Classical methods (10-30) and interpretation were used for the oxytocin tolerance tests

A personal technique (4-27) was used for the exercise tests. The patients were asked to sit up many times from

the horizontal decubitus position until they felt the presence of fetal tachycardia and especially bradycardia after this effort were felt to be manifest of a fetus already compromised in utero

If the exercise and oxytocin tests were repeated simultaneously positive then therapeutic preterm very was indicated even in the absence of meconium in the amniotic fluid. Under these circumstances cesarean section was the procedure of choice

Table III Delivery and perinatal mortality

Groups	Delivery				Perinatal mortality (%)
	Vaginal	No	Abdominal	No	
I (224)	184	82.1	40	17.9	18.2
II (181)	85	47.8	95	52.2	7.7
III (90)	42	46.7	48	53.3	1.1
IV (111)	48	43.3	63	56.7	3.6

RESULTS

Tables I, II and III show data pertinent to perinatal mortality found in the four groups of pregnant patients and the relationship between the weight of new born and the type of delivery

Figures 1-6 show examples of amniocentesis, exercise and tolerance to oxytocin tests performed on the same patients

DISCUSSION

The results in Table I show that perinatal mortality in hypertensive pregnant patients was reduced from 3.2% (group I) to 7.7% (group II) by using the amnioscope and amniocentesis.

This reduction was even more pronounced (1.1%) when in addition to those tests fetal well being was evaluated by monitored maternal exercise and oxytocin tolerance tests (group III).

Since hospital facilities in developing countries usually do not include electronic methods we tried to use clinical methods instead.

This resulted in an increased perinatal mortality from 1.1% to 3.6% (group IV).

However we want to point out that this figure (3.6%) is much lower than that for patients in group (18.2%) in which more accurate tests of fetal well being and maturity were omitted.

Table II shows that the excellent perinatal results obtained in group III were not related to the lower incidence of prematurity; on the contrary it was in this group that the incidence of infants weighing less than 2500 grams was greatest (60.4%). It appeared to be due to the greater accuracy of the electronic methods used in the study which detected the compromised fetus before the excretion of meconium occurred so that even earlier intervention by therapeutic preterm delivery (27-42) was employed.

Finally Table III shows that the incidence of cesarean section was increased in groups II, III and V compared to those in group I. This fact is related to the earlier and frequent finding of chronic fetal distress when amniocopy and amniocentesis (group II) and the maternal exercise and tolerance (oxytocin) tests were used (groups III and IV) and also to the aggravated perinatal risk when vaginal delivery is performed under these circumstances (30-31, 40).

These results are supported by the observations of various authors (1, 17, 19, 35, 37, 38, 43). They all show the relationship of chronic fetal distress, the presence of meconium in the amniotic fluid and emphasize the merits of the amnioscope (7) and amniocentesis (3, 20, 21, 36) for its identification.

The high incidence of changes in fetal heart rate (tachycardia and especially bradycardia) after maternal exercise test and after normal uterine contractions (13, 14, 16, 24, 27, 29, 39, 41) in hypertensive pregnant patients (4, 26, 45) was used for

the early identification of changes in fetal well being in these cases even in the absence of meconium in the amniotic fluid (27-42).

Finally one must take into account that perinatal mortality in high risk pregnant patients justifies preterm therapeutic delivery and a more liberal indication for delivery by cesarean section after the 34th week (44-49).

REFERENCES

- 1 Assali N S. *Biology of Gestation* vol 1 Academic Press New York and London 1968.
- 2 Baird D & Thomson A M. Perinatal mortality. In *Perinatal Problems* (pp 1 and 255) M & S Livingstone Edinburgh and London 1969.
- 3 Behle I, Salomao A J, Santos A U & Neme M. Valor da amniocentese na reducao do obituário perinatal na gravidez complicada por síndrome hipertensiva. *Matern e Inf* 33: 190 1974.
- 4 Behle I, Santos A U & Neme M. Efeitos da Prova de Pose sobre a escuta fetal em gestações complicadas por síndrome hipertensiva. *Matern e Inf* 33: 327 1974.
- 5 Brown J B & Beischer N A. Urinary oestrol excretion as a measure of foetal welfare. In *Fifth World Congress of Gynaecology and Obstetrics* (ed Carl Wood and W A W Walters) p 75. Appleton Century-Crofts New York 1967.
- 6 Browne A L D H & Brennan R K. The application value and limitations of amniocopy. *J Obstet Gynaecol Br Comm* 75: 616 1968.
- 7 De Alvarez R R. Hypertensive disorders in pregnancy. *Clin Obstet Gynecol* 16: 47 1973.
- 8 Dewhurst C J. *Integrated Obstetric and Gynaecology for Postgraduates*. Blackwell Scientific Publications Oxford London Edinburgh Melbourne 1977.
- 9 Dieckmann W J. *The Toxemias of Pregnancy*. The C V Mosby Co St Louis 1957.
- 10 Hammacher K & Hurt M. Fetal heart frequency and perinatal condition of the fetus and newborn. *Gynaecologia* 166: 349 1968.
- 11 Hellman L M & Pritchard J A. *Williams Obstetrics*. Appleton-Century-Crofts New York 1971.
- 12 Hochuli H & Stoeckli A. Schwangerschafts toxikose. Ergebnisse und neue Gesichtspunkte aus 180 Nachkontrollen bei 163 Patientinnen mittels Nierenclearance. *Schweiz Med Wschr* 89: 934 1959.
- 13 Hon E K & Wohlgenuth R. The electronic evaluation of the fetal heart rate. Effect of maternal exercise. *Am J Obstet Gynecol* 87: 361 1961.
- 14 Huntingford P J. Past present and future. In *Perinatal Medicine* (ed P J Huntingford R W Beard F E Hytten & J W Scopes) p 2. S Karger Basel 1971.
- 15 Kaser O, Friedberg V, Oberk G, Thomsen K & Zander J. *Ginecologia e Obstetricia*. Salvat Editores S A Barcelona 1970.
- 16 Kelly J V. Diagnostic techniques in prepartal fetal evaluation. *Clin Obstet Gynecol* 17: 53 1974.

- 17 Kubli F Amniotic fluid and the early detection of fetal hypoxia *In* Perinatal Medicine (ed P J Huntingford K A Hüter & E Saling) p 4 Academic Press New York and London 1969
- 18 Kyank H Epidemiologie der Eklampsie *Wissenschaftl Z Univ Rostock* 15 393 1966
- 19 Lippi U G & Lima G R. Amniocentese Matern e Inf 31 111 1972
- 20 Mandelbaum B Gestational meconium in the high-risk pregnancy *Obstet Gynecol* 42 11 1973
- 21 Mathias L & Neme B Valor da amniocentese na redução do obituário perinatal na gestação complicada com síndrome hipertensivo Matern e Inf 33 425 1974
- 22 Morison J E Patologia Fetal e Neonatal Editorial Pediatrica Barcelona 1972
- 23 Morris W K & McClure Browne J C A Symposium on Non Toxaemic Hypertension in Pregnancy J & A Churchill Ltd London 1958
- 24 Morris N Osborn S B Wright H P & Hart A Effective uterine blood flow during exercise in normal pre-eclamptic pregnancies *Lancet* 2 481 1956
- 25 Neme B Toxemias tardias da prenhez *In* Obstetrícia (ed J Rezende) p 502 Guanabara Kogan 1969
- 26 Neme B Behle I & Santos A U Efeitos da prova de esforço sobre a escuta fetal em gestações complicadas por síndrome hipertensivo Matern e Inf 32 323 1973
- 27 Neme B Behle I & Santos A U Efeitos das provas de esforço e de Pose sobre a escuta fetal Estudo comparativo em gestações complicadas por síndrome hipertensivo Matern e Inf 33 549 1974
- 28 Nesbitt R E L Perinatal Loss in Modern Obstetrics F A Davis Co Publishers Philadelphia 1957
- 29 Pokorny J & Rous J The effect of mother's work on foetal heart sounds *In* Intra Uterine Dangers to the Foetus (ed J Horsky and Z K Stembera) p 354 Excerpta Medica Foundation Amsterdam 1967
- 30 Pose S Castillo J B Mora Rojas E O Sotoyances A & Caldeyro-Barcia R Test of fetal tolerance to induced uterine contractions for the diagnosis of chronic fetal distress *Int J Gynaecol Obstet* 91 1970
- 31 Ray M Freeman R Pine S & Hesselgesser R Clinical experience with the oxytocin challenge test *Am J Obstet Gynecol* 114 1 1972
- 32 Reid D E Ryan K J & Benerschke K Principles and Management of Human Reproduction W B Saunders Co Philadelphia London and Toronto 1972
- 33 Rodrigues Lima J Amnioscopia com novo amnioscópio sólido *Trib Med* 13 14 1970
- 34 Rosa F W International aspects of perinatal mortality *Clin Obstet Gynecol* 13 57 1970
- 35 Ryan D T Ivy R Jr & Pearson J W Fetal bleeding as a major hazard of amniocentesis *Obstet Gynecol* 40 702 1972
- 36 Saling E Das Kind in Bereich der Geburtswissenschaften Thieme Verlag Stuttgart 1966
- 37 Santarelli J Ravina J H & Pinon F 1 Le trouble foetal dans les grossesses pathologiques. Interêt d'examen du liquide amniotique *Gynecol Obstet* 66 293 1967
- 38 Spellacy W N Buhl W C Birk S A & Holzner K K Human placental lactogen levels and the relationship to fetal distress Meconium stained amniotic fluid fetal heart rate patterns and Apgar score A. *Obstet Gynecol* 114 803 1972
- 39 Stembera Z K Fetal tolerance to maternal asphyxia *In* Perinatal Factors Affecting Human Development p 105 World Health Organization Washington 1969
- 40 Stembera Z K The management of fetal distress before and during labor *In* Perinatal Medicine (ed J Huntingford R W Beard F E Hyman & J W Scopes) p 124 S Karger Basel 1971
- 41 Stembera Z K & Hodr J The "Exercise Test" as an early diagnostic aid for foetal distress *In* Intra uterine Dangers to the Foetus (ed J Horsky & Z K Stembera) p 349 Excerpta Medica Foundation Amsterdam 1967
- 42 Stembera Z K Hodr J Brotanek V & Zidovský J Complex early diagnosis of intrauterine foetal distress *In* Intra uterine Dangers to the Foetus (ed J Horsky & Z K Stembera) p 373 Excerpta Medica Foundation Amsterdam 1967
- 43 Strand A Transabdominal isothermal amniocentesis *In* Intrauterine Dangers to the Foetus (ed J Horsky & Z K Stembera) p 398 Excerpta Medica Foundation Amsterdam 1967
- 44 Taylor H C Jr Indicaciones fetales de interrupción del embarazo antes de la vigesimaoctava semana de gestación. Presencia de toxemia específica o hipertensión crónica *Sinopsis Obstet Gynecol* 2 127 1955
- 45 Tchilingirian N G B Fetal monitoring in high pregnancy *Clin Obstet Gynecol* 16 329 1973
- 46 Thalhammer O Patologia Prenatal Salvat Editor SA Barcelona 1970
- 47 Vara P Timonen S & Lokki B Toxaemia of pregnancy A statistical study *Acta Obstet Gynec Scand Suppl* 43 3 1965
- 48 Wallace H M Factors associated with perinatal mortality and morbidity *Clin Obstet Gynecol* 13 107 1970
- 49 Yerushalmy J Relation of birth weight gestational age and the rate of intrauterine growth to perinatal mortality *Clin Obstet Gynecol* 13 107 1970

Submitted for publication Nov 22 1976

Bussâmara Neme
Dept of Obstetrics and Gynecology
University of São Paulo
Brazil

PREMATURE LABOR TREATMENT WITH RITODRINE IN MULTIPLE PREGNANCY WITH THREE OR MORE FETUSES

Joseph Biernarz Niranjana Shah W Paul Dmowski
Ramaa Rao and Antonio Scommegna

*From the Laboratory of Uterine Physiology Department of Obstetrics and Gynecology
Michael Reese Hospital and Medical Center and the Pritzker School of Medicine
of the University of Chicago Chicago USA*

Abstract Modern treatment for anovulatory infertility in the incidence of multiple pregnancies with three or more fetuses and predisposes to prematurity with high perinatal mortality and morbidity. Premature labor was successfully treated in four multifetal pregnancies with ritodrine hydrochloride, a beta mimetic drug relaxing the uterus. Another patient misdiagnosed as false labor was not treated and lost three out of four premature babies. Beta mimetic treatment is indicated in multiple pregnancies even in false labor or when painless progress in cervical dilatation is observed to avoid asymptomatic regression into true labor. In contrast to singleton pregnancies, advanced labor with more than four centimeters cervical dilatation should not preclude good chances for successful treatment. Persistence in treatment and repeated use of the most effective intravenous route combined with oral ritodrine administration is needed because of marked tendency to recurrences of premature labor. Progressive increase in the dose of oral ritodrine may be indicated by decrease in therapeutic response. Maternal tachycardia should be considered as an index of patient responsiveness to the beta mimetic treatment. The therapy is most successful when the patient is hospitalized from the first episode of treatment until at least the 37th week of pregnancy. This is probably less expensive than prolonged hospitalization of several pretermers in an intensive care nursery.

The spontaneous occurrence of multiple pregnancy with three or more fetuses is rare: once in 9800 births for triplets (1) once on 677 000 births for quadruplets and once in 8-20 million births for quintuplets (2). Recently the incidence of multifetal pregnancies increased dramatically due to treatment of anovulatory infertility by agents inducing multiple ovulations: Human Menopausal Gonadotropin (HMG), Human Pituitary Gonadotropins (HPG), Clomiphene Citrate (Clomid) and Human chorionic Gonadotropin (HCG).

The incidence of multiple pregnancy is in the range of 8 to 10% in Clomid treated women (3) while among those treated with exogenous gonadotropins it is as high as 20 to 40% (4). Considering that almost half of the patients in the latter group carry three or more fetuses (5) and that a significant number of these multifetal pregnancies terminate in abortion or premature labor with very high perinatal loss, the fertility drugs have been a mixed blessing to many infertile women.

Prematurity is the single most important cause of the high perinatal morbidity and mortality in multifetal pregnancy. With the rising number of fetuses the degree and incidence of prematurity increase as well. Prematurity occurs in 54% of twins as compared to 9% of singletons (6). The perinatal mortality rates expressed as the number of stillbirths and neonatal deaths per 1000 total births of single, twin, triplet and quadruplet gestations were respectively 39, 152, 309 and 509 (7). The mean length of a twin pregnancy is 37 weeks (8); it is 35 weeks for triplets, 33 weeks for quadruplets (1) and 30 weeks for quintuplets (2). The progressively shorter pregnancy and earlier onset of labor were attributed to the early uterine distension (1). The amount of distension that the uterus will tolerate was found greater in multiparas than in primigravidas whose tendency to prematurity is greater (1).

Known relaxants of the myometrial cell, e.g. beta mimetic drugs, could increase the tolerance of the uterus to distension and delay the onset of labor. Ritodrine hydrochloride, a new beta mimetic drug allegedly has a selective action on the myometrium without producing marked arterial hypotension or tachycardia (9-11).

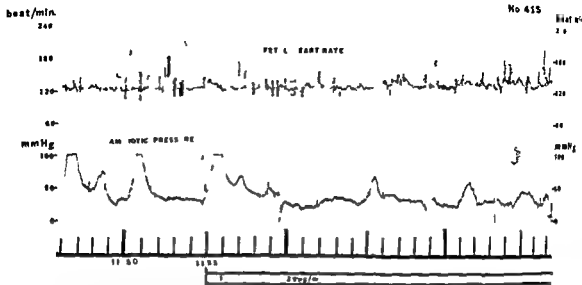


Fig 1 S A G1 P0 in premature labor 3rd week of a quadruplet pregnancy. Marked sensitivity to beta mimetic drugs is indicated by a rapid inhibition of uterine con-

tractions after onset of low rate intravenous nitroder fusion. The uterus relaxed completely in two hours.

The aim of this study was to test the effectiveness of nitroder in delaying the onset of labor in multi fetal pregnancies.

MATERIAL AND METHODS

Nitroder hydrochloride was graciously supplied by Philips Duphar Laboratories Weesp, Holland. Treatment was begun with an intravenous infusion 40 mg/200 ml saline at a very low rate of 40 μ g/min, which was then gradually increased by 40 μ g/min every 10 min, not to 400 μ g/min until adequate uterine relaxation or acceptable side effects occurred (e.g. maternal tachycardia over 130/min or hypotension <90/50 mmHg). The infusion rate was then reduced until side effects disappeared.

Uterine contractility and fetal heart rate were recorded on a Hewlett Packard cardiotocograph Model 8070A. Maternal heart rate and blood pressure were checked and tabulated each 10 min until the effective nitroder dose was established and the uterus was relaxed. There-

after all these signs were checked every two to three hours.

Depending on the established sensitivity to nitroder, infusion treatment was maintained for 17-24 hours, was followed by oral treatment with 10-30 mg (1) tabs 4-8 times daily. The patient was confined to bed during infusion and for the first day of oral treatment. Thereafter limited activity was allowed, but the patient remained hospitalized at least until the end of the 36th week of pregnancy.

CASE STUDIES

Three white women with longstanding primary infertility have been treated with injections of HMG for 18 to 24 days (1 amp=75 IU of FSH and 75 IU of LH) until plasma estradiol has risen to 400-1000 pg/ml or urinary oestrogen to 100-150 μ g/day. At that time an injection of H 10000 IU was administered. Two black women (G2 P1, the other G9) conceived spontaneously without any medication. Multiple pregnancy was suspected at

Table I. Premature labor recurrences in a nitroder treated quadruplet pregnancy (S A G1 P0).

No	Gestation weeks	Cervix		Station	Treatment			
		Dilat.	Effac. %		I.V. (μ g/min)	Duration (h)	Oral (mg q h)	Duration
1	32	1.5 \rightarrow 2	30 \rightarrow 60	-2 \rightarrow -1	25	24	10/3	20 h
2	32	2	40	-2	50	24	10/2	11 h
3	32.5	1.5	50	-2	150	18	10/3	16 d
4	35	2	80	0	Discontinued because of complicating pre-eclampsia			

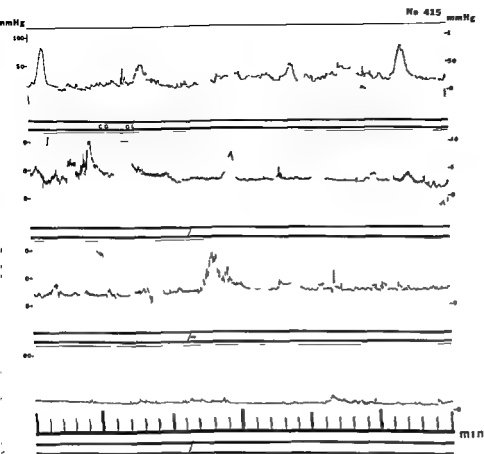


Fig 2 The same patient as in Fig 1 during the third recurrence of premature labor three days later. A higher rate of ritodrine infusion was needed to relax the uterus.

This patient presented three recurrences of premature labor while on oral treatment, controlled by intravenous ritodrine infusion.

because of rapid increase in uterine size incompatible with gestational age. The diagnosis was confirmed by ultrasound in three women during early pregnancy and by X-ray at 30 weeks in the other two women. All women were treated by bedrest with very limited activity (bathroom privileges).

WOMEN TREATED FOR INFERTILITY

Quadruplet pregnancy

S.K., a 27-year-old white woman, was hospitalized at the 32nd week of her pregnancy because of labor pains progressively increasing in frequency and intensity for the

Table II Premature labor recurrences in a ritodrine treated triplet pregnancy (J. S. G1 P0)

No	Gestation weeks	Cervix			Treatment			
		Dilat	Effac ^{cm}	Station	I V (μ g/min)	Duration (h)	Oral (mg q h)	Duration (days)
1	31	0 \rightarrow Ft	0 \rightarrow 50	-3	50-300-200	13	20	2.5
2	31.5	0	30	-3	50-300-00	8	10	3
3	34	0	70	-2	50-200-100	15	10	4
4	36.5	1	80	0	50-200	18	10	3
5	36	Ft	80	0			10	2
6	36	Ft	80	0			10	3
7	37	Ft	80	0			10	3
8	38	3	90	+2	Treatment stopped Onset of labor			7



Fig 3 J S G1 P0 premature labor at the 31st week. Flat plate of abdomen shows three fetuses. On nitrodine treatment pregnancy was continued until term.

preceding 12 hours. The diagnosis of true premature labor was confirmed by a bloody show and progress in cervical dilation in one hour from 1.5 to 2 cm, effacement from 30 to 60% and descent from station -2 to -1 (Table I).

Nitrodine was started with half the usual intravenous infusion rate, 25 µg/min, which was sufficient to cause a complete uterine relaxation in two hours (Fig. 1). After 24 hours infusion was stopped and oral treatment was started with one tablet of 10 mg every 3 hours.

During 20 hours on oral treatment the maternal pulse rate slowed down from 100 to 80 beats/min and uterine contractions reappeared. A second intravenous infusion at 50 µg/min was begun and discontinued after 24 hours.

This was followed by oral treatment 100 mg every 4 hours (Table I). After 11 hours the contractions reappeared and for a third time intravenous infusion was restarted and continued at a higher rate of 150 µg/min for 18 hours. Again uterine contractions disappeared within 1.5 hours from onset of infusion (Fig. 2).

Oral treatment was increased to 200 mg every 4 hours. This dose accelerated maternal pulse to 90 beats/min and maintained the uterus relaxed until the 35th week of pregnancy when labor contractions started again. Because of signs of pre-eclampsia (BP 190/110 mmHg, proteinuria ++ and weight gain) no attempt had been made to stop labor. Seven hours later the patient delivered under light general anesthesia four viable

Table III Premature labor recurrences in a spontaneous triplet pregnancy (M K G2 P1)

No	Gestation weeks	Cervix			Treatment			
		Dilat	Effic %	Station	I V ($\mu\text{g}/\text{min}$)	Duration (h)	Oral (mg q h)	Duration (days)
32	4→6	60→80	-4→-1	200-300 +50 g ethanol 1 h	24	24	20 3	2
32 5	7	70	0	+50 g ethanol 3 h				
33 5	7	90	+1	400-300				
35	9	100	+2	RBCW Onset of labor				
						24	40 3	11

lung 40 min 1st male—vertex by outlet forceps Apgar score 7/10 weight 1575 g 2nd female—by internal podalic version and extraction from transverse lie Apgar score 9 weight 1800 g 3rd male—with a floating head by internal podalic version and breech extraction Apgar score 9 weight 1800 g 4th male—by breech extraction Apgar score 1/9 weight 1800 g

The gestational age of the newborns assessed by Dubowitz score was 35 weeks. The postpartum course was uneventful and the patient was discharged from the hospital days later. All four infants progressed well in special care nursery and were discharged after three weeks when their weight reached 2000 g.

Triplet pregnancy (Fig 3)

S is a white 27-year-old G1 P0 who was hospitalized at 31 weeks of gestation with painful regular contractions of increasing frequency and intensity. True premature labor was recognized by progress from a long closed and anterior cervix to a fingerup open and 50% effaced during one hour. Intravenous administration of nitrodine was started at increasing rates from 50 to 300 $\mu\text{g}/\text{min}$ which had to be reduced to 200 $\mu\text{g}/\text{min}$ due to marked maternal pulse acceleration from 90 to 144/min. Uterine contractility was completely inhibited in 2.5 hours; the infusion continued 13 hours. On oral treatment 20 mg every 4 hours the patient was gradually mobilized during 2.5 days. Similarly as in quadruplet pregnancy there were several recurrences of premature labor when the patient was on oral treatment. They are summarized in Table II. Starting with the second recurrence intravenous nitrodine infusion was combined with oral treatment which was maintained throughout the infusion period. This combined administration marked on Table II by + between intravenous and oral treatment was well tolerated except for transient palpitations immediately after nitrodine in

for elevated uric acid levels (8.3 mg%) at 38 weeks. No signs of pre-eclampsia were observed.

At 37 weeks oral treatment has been discontinued. At the 38th week 8 weeks from onset of treatment labor started (Table II) and progressed smoothly to a spontaneous delivery of three infants at 28 min each from vertex presentation under pudendal block anesthesia. 1st male—Apgar score 9/10 weight 2290 g 2nd female—Apgar score 8/10 weight 2300 g 3rd female—Apgar score 7/10 weight 2170 g.

The Dubowitz score of the newborns was 37 to 38 weeks. The neonatal course was without complications.

Quintuplet pregnancy (premature labor not treated with nitrodine)

E I is a 26-year-old white woman who was hospitalized at the 27th week of pregnancy with a term size uterus because of a mucous and watery discharge without pains. Negative nitrazine and fern tests excluded ruptured bag of water. No uterine contractions were recorded; cervix was 1.5 cm dilated and 50% effaced and did not progress during 48 hours. The woman has not been treated with nitrodine because the diagnosis of true premature labor could not be established. She has been alerted to report immediately uterine contractions. The patient disregarded mild discomfort until pains became strong and membranes ruptured. The cervix was found six centimeters dilated 50% effaced station +2. She delivered within one and a half hours under pudendal anesthesia. 1st female—vertex NSVD Apgar score 3/7 945 g 2nd anophthalmic monster—of the same amniotic sac Apgar score 0/0 157 g 3rd male—spontaneous breech Apgar score 4/6 845 g 4th male—assisted breech Apgar score 3/6 960 g 5th male—breech extraction Apgar score 3/6 900 g.

The condition of the first and fifth baby was poor that of the third and fourth was fair. All four developed respiratory distress syndrome with repeated periods of apnea and bradycardia treated with continuous positive airway pressure (CPAP) and oxygen resuscitation. Only the first baby survived treated with Ampicillin Kanamycin and blood transfusions. The baby was discharged after 70 days of hospitalization in good condition weighing 1900 g.

Spontaneous triplet pregnancies conceived without fertility treatment

M K is a 21-year-old black G2 P1 who was hospitalized at the 32nd week of pregnancy because of premature labor

recognized by rapid progress in cervical dilatation from 4 to 6 cm effacement from 60–80% and descent from -4 to -1 station in one hour although no labor pains were felt by the patient. Uterine contractions of increasing frequency were recorded five to two minutes apart. Ritodrine 1 V infusion was started and increased to 300 µg/min spacing uterine contractions 10–12 min apart. Complete uterine relaxation was attained with the additional ethanol treatment 50 g in 1000 cc 1/2 dextrose 1/4 M saline infused in one hour and repeated during the following three hours (Table III).

No further progress in labor has been observed and the combined intravenous infusion has been discontinued after 24 hours. Oral ritodrine 20 mg every three hours has been started and had to be increased two days later to 30 mg every three hours (Table III) because maternal pulse slowed down from 170 to 80/min.

No signs of pre-eclampsia have been observed. Urinary 24 hour estron excretion increased from 22.2 to 39.8 mg/24 hours between 37th and 38th 1/2 weeks. At that time regular contractions every 3–4 min started again. Cervix was found 7 cm dilated and 90% effaced with the head engaged +1 (Table III). A repeat intravenous ritodrine infusion had to be increased to 400 µg/min to inhibit uterine contractility again. It was reduced to 300 µg/min and was maintained during 24 hours but the cervix remained 7 cm dilated. The patient was kept in absolute bedrest in Trendelenburg position. Difficulties in complete bladder evacuation forced us to grant the patient bathroom privileges.

At the 35th week of pregnancy the BOW ruptured with 9 cm dilatation 100% effacement and station +2. The patient delivered in 1 hour 51 min under pudendal anesthesia with a mediolateral episiotomy. 1st male—spontaneous vertex. Apgar score 6/7 weight 2220 g. 2nd male—spontaneous breech. Apgar score 6/7 weight 2140 g. 3rd male—spontaneous vertex. Apgar score 4/6 weight 1880 g.

All babies were discharged 9 days later in good condition.

M H was a 46 year-old G9 P8 with triplets diagnosed by ultrasound at the 23rd week of pregnancy (Fig. 4a, b). She was hospitalized at 33 weeks in premature labor. In spite of ethanol treatment the cervix dilated from 1 to 1.5 cm effaced from 30 to 60% and the station changed from -3 to -1. Ritodrine infusion 250 µg/min stopped further progress of labor and was substituted after 24 hours by oral ritodrine 20 mg q 4 hours. The dose had to be increased to 30 mg q 3 hours at the 35th week because the maternal heart rate tended to descend from 110 to 80 beats/min. This treatment was well tolerated and continued until the 38th week when amniocentesis confirmed fetal maturity. L/S ratio 3/3 orange cells 20% and creatinine 1.8 mg/dl. Ritodrine treatment was stopped two days later membranes ruptured and labor started. Three female newborns were delivered spontaneously in 2 hours and 15 min from vertex presentations with Apgar score of 9/9 each and weights of 1830 g, 2380 g and 2385 g respectively. Except for transient hypoglycemia of the first small gestational age infant the neonatal course of these near term babies was uneventful.

COMMENTS

Ritodrine hydrochloride treatment has been successful in delaying the onset of true premature labor in four multifetal pregnancies with salvage of infants. In another woman who did not present definite signs of labor at the 27th week we missed right time for early treatment with loss of three of four babies. In the quadruplet pregnancy began at 31 weeks of gestation however the delivery was postponed with ritodrine treatment until 35th week. It is conceivable that even further of labor could have been achieved in this case. However we elected to discontinue treatment because of complicating pre-eclampsia. In three pregnancies labor began at the 31st week delivery was postponed until term in two and at the 35th week in one. There was no perinatal mortality in ritodrine treated patients although a 31% in triplets and 51 percent in quadruplets be expected (7). Delivery occurred 2 to 3 weeks later than could be anticipated on the basis of number of carried fetuses (1).

One major problem in assessing the efficacy of treatment in premature labor was the difficulty in distinguishing between true and false labor. If false labors were included into the study group it would bias the results because uterine contractions occur even without treatment. In singleton pregnancies a basic requirement for including a patient into ritodrine treatment is a confirmed progressive cervical effacement, dilatation and descent of the presenting part (9) generally considered as proof of a true labor. On the other hand in multifetal pregnancies it is much more difficult to distinguish between true and false labor because cervical dilatation, effacement and descent of the presenting part may be observed long before onset of labor. The genital tract is

Fig. 4a M H G9 P8 23rd week of pregnancy. Longitudinal ultrasound scan reveals two fetal heads (1 & 2). S = Symphysis U = Umbilicus P = Placenta. Amniotic fluid. Polyhydramnios is present.

Fig. 4b Longitudinal ultrasound scan 3 cm to the right of the midline in the same woman visualizes a third fetus in breech footling presentation (h = fetal heart). Weeks of pregnancy premature labor was stopped with ritodrine treatment and pregnancy continued until 38 weeks. From A. Cadkin and M. Motew. Clinical Atlas of Scale Ultrasonography in Obstetrics. Charles C. Thomas, Springfield (with authors & publishers permission).



pared is predisposed to an almost asymptomatic progress into an irreversible active phase of labor as in our case of quintuplets and in one of our triplets (M. K.).

In multiple pregnancy it is safer to treat a false premature labor unnecessarily than to overlook an asymptomatic progress of labor risking early prematurity with its high perinatal mortality and morbidity. This is especially important in women who conceived after longstanding infertility, and who are desperate to have living and healthy children.

The observations collected in the second triplet pregnancy (M. K.) suggest that criteria of failed treatment accepted for singleton pregnancy i.e. cervical dilatation over 4 cm and marked effacement 80% are not valid in multiple pregnancies. The marked uterine distension makes the diagnosis of labor more difficult. On the other hand it makes possible a prolonged delay of an advanced labor even though uterine contractions are not completely inhibited. Absolute bedrest in Trendelenburg position indicated in these cases may be difficult to enforce because of the breathing difficulties and voiding discomfort.

A prolonged hospitalization almost until term is indicated by the marked tendency to recurrences necessitating immediate repeating of intravenous treatment. This can be assured only if the patient is hospitalized. It is less expensive and more rewarding to keep a mother hospitalized for several weeks than to treat several prematures in an intensive care nursery for prolonged periods of time.

Combined intravenous and oral nitroglycerine administration seemed to be most successful in inhibiting recurrent uterine contractility (see Table II). Close monitoring of maternal pulse on oral treatment helped to reduce the incidence of recurrences. The initial marked response to nitroglycerine decreases with time necessitating progressively increasing doses to maintain the uterus relaxed. Maternal heart rate is a good indicator of the changing sensitivity to beta mimetic drugs. Whenever the maternal pulse decreases below 80/min a recurrence of premature labor may be expected. By increasing the oral nitroglycerine dose a positive chronotropic effect takes place with tachycardia of 90-120/min and generally efficient inhibition of the uterine contractility.

Finally alcohol a known inhibitor of oxytocin release may be combined with the relaxing effect of beta mimetic drugs on the myometrial cell in a

combined multi target approach for prevention and treatment of premature labor (17).

Preparations for multiple delivery consist in organizing well in advance an alert emergency team available at any time. It consists of obstetricians, neonatologists (one for each expected newborn), anesthesiologists and nurses. An adequate obstetrical instrumentation, warmers, resuscitation equipment and blood must be ensured.

The conduct of labor aims at reducing the mechanical stress on the premature baby's head during its passage through the genital tract, maintaining an intact bag of water until full dilatation is reached, application of outlet forceps and episiotomy. Prevention of postpartum hemorrhage is indicated by manual intrauterine exploration checking of cervical and vaginal lesions and judicious use of oxytocics. If labor does not progress smoothly or complicated obstetrical operations are envisioned delivery by cesarean section is advisable.

Although these preliminary observations need further confirmation on a larger material they are of basic interest in view of increasing incidence of multifetal pregnancy in modern obstetrics and the limited modalities of treatment currently available (5).

ACKNOWLEDGEMENTS

This study was supported by N. V. Philips Dr. Weesp, The Netherlands and Michael Reese Medical Research Institute Council.

REFERENCES

- McKeown T & Record M G Observations on foetal growth in multiple pregnancy in man. *J. E. J. Child* 386 1957
- Berbo J N, King B & Janusz A Quintuplets pregnancy. *JAMA* 188 813 1964
- Hack M, Brish M, Serr D, Insler V, Salom M & Luenfeld B Outcome of pregnancy after induced ovulation follow up of pregnancies in children born after clomiphene therapy. *JAMA* 213 1379 1977
- Thompson L & Hansen L Pergonal (Mestropin) A summary of clinical experience in the induction of ovulation and pregnancy. *Fertil Steril* 21 1970
- Jewelwicz R Management of infertility resulting from anovulation. *Am J Obstet Gynecol* 127 9 1975
- Friedman G A & Little W A An evaluation of management of twin delivery. *Bull. Sloane Hosp.* 39 1958

- Friedman ■ A & Little W A The twin delivery Factors influencing second twin mortality *Obstet Gynecol Survey* 13 611 1958
- Gutmacher A F & Kohl S G The fetus of multiple gestations *Obstet Gynecol* 12 578 1958
- Wesselius-deCasparys A Thierry M Yo Le Sian A Baumgarten K Brossens J Gamisans O Stolk T ■ & Vivier W Results of double blind multicentre study with ritodrine in premature labor *Br Med J* 2 144 1971
- Bieniarz J Motew M & Scommegna A Uterine and cardiovascular effects of ritodrine in premature labor *Obstet Gynecol* 40 65 1972
- Bieniarz J Ivankovich A & Scommegna A

Cardiac output during ritodrine treatment in premature labor *Am J Obstet Gynecol* 118 910 1974

- 1° Bieniarz J Combined multitarget approach to prevention of premature labor *In* *Premature Labor: Its Management and Therapy* (ed F P Zuspan) *J Reprod Med* 9 93 1972

Submitted for publication Feb 27 1977

Joseph Bieniarz
Department of Obstetrics and Gynecology
Michael Reese Medical Center
Chicago Illinois 60616
USA



THE THERMOGENIC ACTIVITY OF EXOGENOUS E AND F PROSTAGLANDINS IN HUMANS

James R Dingfelder and William E Brenner

From the Department of Obstetrics and Gynecology University of North Carolina Medical School Chapel Hill North Carolina 27514 USA

basic understanding of the physiologic effects of the prostaglandins on the human reproductive system has been one of the diverse scientific interests of Professor Axel Ingelman Sundberg (19). Although the obvious clinical utility of these ubiquitous compounds directed early attention to their therapeutic aspects more recently there has been renewed interest in the basic physiologic mechanisms of the prostaglandins in reproductive medicine. Undoubtedly the work of Dr Ingelman Sundberg has stimulated many of his colleagues students and fellow scientists to pursue such basic studies in greater detail.

The reporting of thermal (thermogenic) side effects after administration of prostaglandin to humans has received scant mention in the general literature. Although pyrexia of various degrees is occasionally mentioned it has usually been considered as a possible indicator of morbidity presumably secondary to endometritis or other infection. There have been few reports on the thermogenic effects of exogenous prostaglandin of various types given by several routes in human subjects. Since prostaglandins appear to be intimately involved in physiological and pathological temperature regulation presumably both on a central nervous system and peripheral circulatory agents a closer inspection of temperature changes after prostaglandin administration appears warranted.

MATERIALS AND METHODS

Extensive observations were made on 194 subjects between 18 and 47 years of age and 8 and 27 menstrual weeks gestation who were aborted in the Clinical Research Unit of North Carolina Memorial Hospital Chapel Hill North Carolina (USA). Particular attention was directed to the following clinical observations:

- 1 Hourly oral temperature
- 2 Presence of visible shivering
- 3 Presence of endometritis and other infection
- 4 Subjective feelings of cold or hot

All subjects received intramuscular prostaglandin injections according to the dose schedules outlined in Table I. Since previously reported (4) experience had mentioned possible thermogenic side effects prophylactic medications were given in various combinations to certain subgroups to attenuate these and other side effects as outlined in Table II. Only group E3c subjects who had received the PGE₂ analogue also received aspirin. No subjects who had been treated with PGF_{2a} analogue received aspirin since only the E analogue had been reported to have been associated with pyrexia.

15(S)-15 methyl prostaglandin E₂ methyl ester in an original concentration of 2 mg per milliliter was diluted with normal saline to a concentration of 15 µg per milliliter while 15(S)-15 methyl PGF_{2a} Tham salt was given in

Table I Groupings of patients receiving various doses of 15 methyl prostaglandin E₂ and F_{2a} analogues

Group	Subjects	Dosage	Medications
I 15(S)-15 methyl PGE₂ methyl ester (1 M)			
E1	20	5 µg q 4 h	-
E2	10	10 µg q 8 h	-
E3a	31	10 µg q 4 h	-
E3b	36	10 µg q 4 h	10 mg diazepam 1 M 1 h before induction
E3c	32	10 µg q 4 h	Concomitant medications (see Table II)
E4	5	25-50 µg q 8 h	-
II 15(S)-15 methyl PGF_{2a} methyl ester (1 M)			
F1a	70	250 µg q 2 h	-
F1b	40	250 µg q 2 h and cervical laminaria	Concomitant medications (see Table II)

Table II Concomitant prophylactic medications given to selected subgroups of patients

I Group E3c

A Prochlorperazine

- 1 10 mg IM one hour before induction
- 2 25 mg rectal suppository every six hours after induction until abortion

B Lomolil (Searle brand of diphemoxylate HCl 2.5 mg and atropine sulfate 0.025 mg per tablet)

- 1 4 tablets one hour before induction
- 2 2 tablets every four hours after induction until abortion

C Acetylsalicylic acid

- 1 1.3 g po one hour before induction
- 2 300 g rectal suppository every four hours after induction until abortion

II Group F1b

A Prochlorperazine—same as above

B Lomolil—same as above

C Acetylsalicylic—none given

one milliliter undiluted doses. Administration was by deep gluteal muscle injection. These were no significant differences in the ages, parity and duration of gestation among the groups treated with either analogue.

RESULTS

Shivering

Table III summarizes the observed incidence of visible shivering (rigors) noted in subjects receiving the prostaglandin analogues. The E4 subgroup (5 subjects) were the initial patients treated by the authors with either analogue. The first 2 patients received 60 µg doses of 15 methyl PGE₂ analogue. The remaining 3 patients received 25 µg doses. All five patients exhibited profound rigors within a few minutes of the prostaglandin injection much to the surprise of the investigators since the previous experience of Karim (14) with similar doses had not emphasized this side effect.

Subsequent patients treated with the same PGE analogue received reduced amounts of the compound both in order to have fewer side effects such as shivering and to ascertain the lowest effective dosage. The group which received the lowest dose (2 µg) had the lowest incidence and mean number of visible rigors (65%). Of these patients given a 10 µg dose the group receiving the entire list of concomitant prophylactic medications (E3b) had a similarly lower (66%) incidence of rigors. The remaining patients receiving the 10 µg dose without premedication had similar higher percentages of

shivering (77–83%). The vast majority of these episodes occurred within 15 min after prostaglandin injection. There was no tendency for the phenomenon of rigor to increase or decrease with subsequent injections. Covering patients with blankets reduced the severity of shivering even though the patients' oral temperatures remained elevated. Removal of blankets usually brought a return of symptoms.

No patient who received a PGF analogue was ever observed to exhibit shivering.

Subjective temperature sensations (Table IV)

A subjective feeling of coldness accompanied visible rigors in all patients thus affected and was occasionally mentioned by patients who were not visibly shivering. Subjective cold was experienced by 93% of all patients receiving PGE analogues while 75% were observed to have shivering. The complaint of coldness like that of shivering occurred shortly after the prostaglandin injection and was attenuated by covering the patient with blankets. There appeared to be no correlation between the extent of oral temperature elevation and the onset or duration of subjective sensations of coldness.

Those patients who received the PGF analogue had a low incidence of subjective coldness (17%) but a significantly higher tendency to mention sensation of excessive warmth or flushing (40%) than the E subgroups (12%).

Fever (Table V)

Periodic elevations of oral temperature above 38°C were regularly related to injections of PGE analogues were evident in the initial patient group tested (E1) and are depicted in Fig. 1. These patients received

Table III Incidence of visible shivering (rigors) among subgroups of patients receiving 15 mg PGE₂ or PGF_{2a} analogues

Subgroups	No.	%	Mean no. episodes	Range
E1	13	65	1.2	0–3
E2	8	80	1.4	0–4
E3a	24	77	1.6	0–5
E3b	30	83	1.1	0–7
E3c	21	66	1.3	0–5
E4	5	100	1.6	1–3
F1	0	0	0	0
F2	0	0	0	0

Table IV Subjective temperature sensations experienced by patients receiving 15 methyl PGE₂ or F2a analogues

Group	Feels hot		Feels cold	
	No	%	No	%
E	2	10	11	90
	0	0	10	100
	5	16	27	87
	7	5	36	100
F	2	6	29	91
	0	0	5	100
	8		93	
	9	45	3	15
Total E		38	10	
Total F		40	17	

or 50 µg doses exhibited maximal fevers at 1-3 hours after each injection. All temperatures returned to pretreatment levels in 4-6 hours and remained there unless subsequent injections were administered. Patients experienced coldness and shivering while their temperature was rising but did not experience coincident shivers or subjective cold feelings at times of maximal temperature elevation. In Fig. 2 the effect of the same 10 µg dose of 15 methyl PGE₂ given at 4-8 hour intervals is depicted. In all cases there is a prompt temperature rise associated with each injection time. Injections given at four hour intervals appeared to be too frequent to result in a return to pretreatment temperatures

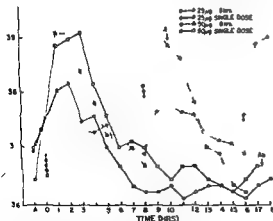


Fig. 1 Mean hourly oral temperature in patients receiving intramuscular injections of 15(S) 15 methyl prostaglandin 2 analogue (A = admission temperature). Note consistent elevation after each injection (†)

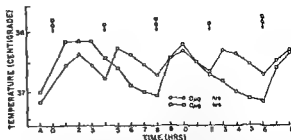


Fig. 2 Mean hourly oral temperatures of patients receiving 10 µg intramuscular injections of 15(S) 15 methyl prostaglandin E₂ analogue at four or eight hour intervals. Note consistent elevation after each injection (†)

while the mean temperature had receded to its basal level in patients injected at eight hour intervals.

Patients receiving the PGF analogue (Table V) did not demonstrate any significant overall temperature elevation although several cases of endometriosis (see Table VI) contributed to a slight mean temperature elevation.

Effect of concomitant prophylactic medications

The patients in group E3c also received acetylsalicylic acid (aspirin) throughout the induction-to-abortion interval. Aspirin appeared to exert an antipyretic effect since this group of patients had the lowest mean hourly temperatures and the smallest percentage of patients (53%) with temperature exceeding 38°C at any time during the treatment period. Thus antipyretic effect was best demonstrated in patients of groups E3a, b, c who received an identical 10 µg dose at four hour intervals (Fig. 3). Patients receiving aspirin had a lower mean temperature although at times the differences were not

Table V Incidence of oral temperature greater than 38°C among patients receiving 15 methyl PGE₂ or PGF_{2a} analogues

Subgroups	No %		Fever	
			Maximum	Mean
E1	1	60	38.4	38.1
E2	8	80	39.2	38.4
E3a	26	87	40.2	38.2
E3b	24	67	41.0	38.2
E3c	17	53	38.7	37.8
E4	5	100	39.1	38.3
F1	3	15	38.7	37.4
F2	10	25	38.9	37.6

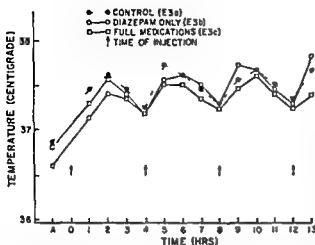


Fig 3 Mean hourly oral temperature of patients receiving 10 µg intramuscular injections of 15(S) 15 methyl prostaglandin E₂ analogue at four hour intervals. Controls received no additional medications while full medications (E3c) group received aspirin, prochlorperazine and Lomotil as outlined in Table II

significant. Fig 4 shows that the medications exerted no significant effect on the abortion time or cumulative abortion rates in these same three subgroups of patients (E3a, b, c). The beneficial effect of prophylactic prochlorperazine and Lomotil for antiemetic and antidiarrheal activity has been reported elsewhere (10).

Endometritis

The incidence of endometritis was low throughout the study except in patients who had cervical laminaria inserted at the time of their initial prostaglandin insertion (group F2). A 10% incidence of endometritis may explain the 25% incidence of febrile episodes noted in these patients.

Table VI Incidence of endometritis in patients receiving 15 methyl PGE₂ or PGF_{2a} analogues

Subgroup	No	%
E1	1	5
E2	0	0
E3	0	0
E3a	0	0
E3b	0	0
E3c	0	0
E4	0	0
F1	1	5
F2	4	10

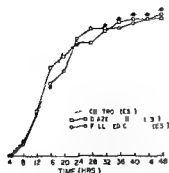
Table VII Fever associated with E prostaglandins

Reference	Incidence (%)	Max temp	Dosage
Intravenous PGE₂			
Naismith (18)	40	-	0.25-8.0 µg/min
Karim (15)	2	-	5 µg/min
Karim & Filshie (16)	1*	38°C	5-7.5 µg/min
Vaginal PGE₂			
Henzl (11)	■	-	0.5-3.0 mg (vag solution)
Jones (13)	70	39.5°C	70 mg vag supp q 2-4 h
Lauerson (17)	70	40.0°C	70 mg vag supp q 2-4 h
Freid (9)	100	38.5°C	70 mg vag supp q 2-4 h
Schulman (20)	33	-	70 mg vag supp q 2-4 h
Bolognese (3)	55	40.5°C	70 mg vag supp q 2-4 h
Corson (6)	50	39.7°C	70 mg vag supp q 2-4 h
Southern (?)	45	-	70 mg vag supp q 3-5 h
Extraovular 15 me PGE₂			
Choo (5)	4	38.9°C	25 µg single dose
Intra-amniotic PGE₂			
Hudson (12)	12	-	10 mg single dose
Intra amniotic 15 me PGE₂			
Amy (1)	30	-	100 mg single dose
Intramuscular 15 me PGE₂			
Ballard (2)	100	-	10 mg q 2 h
Karim (14)	12	-	25-50 mg q 8 h
Gruber (10)	93	-	10 mg q 4 h
Brenner (4)	65	-	5 mg q 4 h

DISCUSSION

Although the subject of temperature elevation due to non infectious side effects of prostaglandins for abortion has not been addressed specifically in the literature of human clinical studies, most authors have mentioned widely variant incidences of febrile complications associated with administration of E prostaglandins.

Table VII is a summary listing of several authors which mentioned the percentage of patients who had fever in studies utilizing E prostaglandins and their analogues for abortion or term labor induction. The disparity of cited observations is striking among studies with identical or nearly identical dosages and routes of administration. Presumably most investigators have learned that febrile episodes associated with exogenously administered prostaglandins are transient and without morbid



Cumulative abortion rates of patients receiving intramuscular injections of 15(S) 15 methyl prostaglandin E₂ analogue in four hour intervals. The groups had different schedules of prophylactic medications listed in Table II

It may be possible that such febrile episodes were ignored or otherwise under reported. It is difficult to explain two- or three- differences in cited incidences of fever using same dosages and types of prostaglandins. One must however be cautious in dismissing fever in cases since morbid fevers of endometritis or other infectious origins may be temporarily masked thus delaying appropriate treatment. Although the maximum oral temperature recorded by the authors cited in Table VII was 40.5°C, temperatures as high as 42.5°C have been reported in preliminary studies utilizing high dosages of 15 methyl PGE₂ analogue. Such fever if sustained might prove injurious. Fortunately effective dose schedules now require only a fraction of those which precipitated fevers of the degree just noted.

The precise pathophysiologic events which lead to fever production after E prostaglandin use have not been elucidated. The initial observation that pyretics are often also inhibitors of prostaglandin synthesis was an early clue to the association.

Animal studies (8) in which prostaglandins of E type when administered into the lateral ventricle or hypothalamus provoked a febrile response gave support to a hypothesis that temperature changes were of central origin perhaps involving an upward displacement of the threshold for central thermo-sensitivity (22). Whether or not this is a direct prostaglandin effect or is mediated by increased production of catecholamines remains speculative. It is more certain that pyrogen induced fever is associated with production of prostaglandins centrally since cerebrospinal prostaglandin levels rise during endotoxin fever (7).

It is also possible that some of the thermal side effects, especially the visible shivering and subjective feeling of coldness may have both central and peripheral components in so far as the action of prostaglandin is concerned. Consistently our own observations revealed that the initial symptom seen usually within minutes after injection of the 15 methyl PGE₂ analogue was that of a cold sensation followed shortly by visible shivering. These symptoms appeared to be related to surface heat loss since they could be prevented or significantly ameliorated by covering the patients with blankets and would reappear upon removal of the warm covering. An attractive hypothesis to explain these observations would be that the peripheral vasodilating activity of the E prostaglandin promoted surface heat loss from the subjects thus provoking the initial symptomatology. Significant temperature elevation on the other hand was never observed until at least 1-2 hours after prostaglandin injection by which time little or no shivering was present. It was also interesting to observe that venous blood sampling became progressively more difficult as time progressed after each injection leading to the speculation that a compensatory veno-constriction had evolved promoting heat retention and temperature elevation. Such an hypothesis may merit future testing.

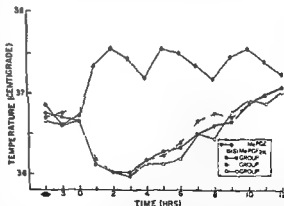


Fig 5 Comparison of mean hourly oral temperatures in patients receiving 15(S) 15 methyl prostaglandin analogues E₂ or F_{2a}. Patients in Group I and those receiving the E₂ analogue had no additional medications. Group II patients received prophylactic prochlorperazine and Lomotil (in dosages identical to those of Table II). Group III patients had lamaria inserted in addition to receiving prochlorperazine and Lomotil. No patient in any of the subgroups received aspirin.

The E prostaglandins appear to evoke the most consistent and marked temperature elevation in the several animal species studied thus far. In contrast F prostaglandins elicit no such rise. Our own observations in a previous report (10) illustrated in Fig 5 show an opposite effect, i.e. a transient and significant temperature decline for up to 8 hours after administration of 15(S) 15 methyl PGF_{2a}.

CONCLUSION

Administration of E prostaglandins and their analogues by differing routes and dose schedules provoke thermal side effects such as temperature elevation, rigors and subjective coldness in a high percentage of subjects. In 99 patients having abortion induced with intramuscular 15 methyl PGE₂ analogue, the concomitant administration of acetyl salicylic acid appeared to reduce the incidence and degree of these side effects. The severity of such side effects appears to be dose related. Although the E prostaglandins and their analogues appear to be thermogenic compounds exerting their effects through a centrally mediated mechanism, there may also be peripheral vascular actions of these prostaglandins which contribute to the appearance of thermal side effects.

ACKNOWLEDGEMENT

Supported by United States Public Health Service Research Grant RR46 from the General Clinical Research Centers Branch, Division of Research Resources and supported in part by a grant from the International Fertility Research Program, Research Triangle Park, North Carolina (AID/csd 2979).

REFERENCES

- 1 Amy J, Karim S M M & Swasamboo R. *J Obstet Gynecol Br Comm* 80: 1017 1973.
- 2 Ballard C & Quilligan E. *Contraception* 9: 523 1974.
- 3 Bolognese R J & Corson B L. *Am J Obstet Gynecol* 120: 281 1974.
- 4 Brenner W E, Dingfelder J R, Staurowsky L G, Kumarasamy T & Grimes D A. *Am J Obstet Gynecol* 120: 833 1974.
- 5 Choo H T, Cheng P, Yam K L & Karim S M M. *In* *Obstetrical and Gynaecological Uses of*

- Prostaglandins* (ed S M M Karim) vol 1 1974. Asian Federation of Obstetrics and Gynecology, Proceedings of the 1st Inter-Congress Singapore April 1976.
- 6 Corson B L & Bolognese R J. *J Reprod Med* 169 1974.
 - 7 Feldberg W, Gupta K P, Milton A S & Landt S. *J Physiol* 234: 279 1973.
 - 8 Feldberg W & Milton A S. *In* *The Pharmacology of Thermoregulation* (ed P Lomax and E. S. Baum) pp 307-310 Karger, Basel 1973.
 - 9 Freid M D, Tredway D R & Mishell D R. *Contraception* 8: 255 1973.
 - 10 Gruber W, Brenner W E, Staurowsky L G, Dingfelder J R & Wells J S. *Fertil Steril* 27: 19 1976.
 - 11 Henzl M H, Noriega L, Aznar R, Ortega E, Segre E. *Prostaglandins* 1: 205 1971.
 - 12 Hudson I, Melville H A H & Ruess C F. *Obstetrical and Gynaecological Uses of Prostaglandins* (ed S M M Karim) vol 1 pp 205-209 L. Federation of Obstetrics and Gynecology Proceedings of the 1st Inter-Congress Singapore April 1976.
 - 13 Jones J R, Perez R J & Bienart W. *Prostaglandins* 7: 149-167 July 1974.
 - 14 Karim S M M, Sharma S D & Filshie G M. *In* *The Prostaglandins: Clinical Applications in Human Reproduction* (ed E Southern) pp 307. F. Publishing Company, Kisco, New York 1972.
 - 15 Karim S M M & Filshie G M. *Br Med J* 1: 1970.
 - 16 Karim S M M & Filshie G M. *J Obstet Gynecol Br Comm* 79: 1 1972.
 - 17 Lauersen N H, Secher N J & Wilson A. *Am J Obstet Gynecol* 122: 947 1975.
 - 18 HarSmith W, Barr W & Macvicar J. *J Obstet Gynecol Br Comm* 80: 531 1973.
 - 19 Sandberg F, Ingelman Sundberg A & Ryden C. *Acta Obstet Gynecol Scand* 43: 85 1974.
 - 20 Schultman H, Saldana L, Tsai T, Leberman T, Cunningham M & Randolph D. *Prostaglandins* 195 1974.
 - 21 Southern E M. *In* *Obstetrical and Gynaecological Uses of Prostaglandins* (ed S M M Karim) 105-118. Asian Federation of Obstetrics and Gynecology Proceedings of the 1st Inter-Congress Singapore April 1976.
 - 22 Stitt J T, Hardy J D & Stolwijk J A I. *Am J Physiol* 227: 677 1974.

Submitted for publication Jan 21 1977

William E Brenner
Department of Obstetrics and Gynecology
The University of North Carolina at Chapel Hill
Old Clinic Building 226 H
Chapel Hill NC 27514
USA

THERAPEUTIC ABORTION IN AN OUT PATIENT CLINIC

A Prospective Investigation of Complications and Patient Acceptability

Birger H. Møller Jørgen Trær Hansen Poul Diederich and Viggo Oram

From the Surgical Out patient Clinic Aarhus Kommunehospital Aarhus Denmark

Abstract The incidence of complications associated with therapeutic termination of pregnancy was analysed in 1349 patients whose average age was 26.3 years. Of the patients 51.8% were 25 years or under 56.3% were seen before the 8th week of pregnancy and 17.4% had previously had one or more legal abortions. All the operations were performed under local anaesthesia and by the same doctor who was an experienced gynaecologist. Complications associated with the operation occurred in 14 patients (1%) viz infections in the uterus and tubes 9 cases incomplete abortion necessitating repeat curettage 1 case and profuse bleeding requiring blood transfusion 1 case. No uterine perforations or cervical ruptures were encountered.

Questioned about subjective discomforts of the operation 74% of the women answered that the discomfort was considerable whereas 67.6% described it as only slight 4.8% would have preferred general anaesthesia.

We conclude that therapeutic abortion can be performed safely and with an acceptably low incidence of complications in out patients provided the operations are performed by an experienced gynaecologist.

In 1973 a revision of the Danish law concerning pregnancy gave all women the right to free abortion before the end of the 12th week of pregnancy. Since then a steep increase in the number of terminations of pregnancy has occurred (Fig. 1).

Up to that time almost all legal abortions were performed under general anaesthesia during admission to a hospital. However the increasing number of abortions caused great difficulties for the gynaecological departments. In our hospital we have tried to overcome these difficulties by performing the operations on out patients and under local anaesthesia. This policy might be expected to be followed by an increasing number of complications and therefore a prospective study of

such complications was undertaken. The experience gained during the first 2 years of the period is reported below.

MATERIAL AND METHODS

During the two-year period from Jan. 1 1974 to Dec. 31 1975 a total of 1391 women were referred to our hospital for therapeutic abortion. Abortion must be performed before the end of the 12th week of pregnancy reckoned from the first day of the last menstrual period. Termination of pregnancy was performed in 1349 of the patients. Abortion was refused on account of the duration of pregnancy in 21 cases 9 patients were not pregnant 10 changed their minds and 2 had a spontaneous abortion before the operation.

All the women were referred to the hospital by their general practitioners. After history taking each patient was subjected to physical examination a complete blood count and blood grouping. In addition Papanicolaou smears and smears for gonorrhoea were routinely prepared. All the operations were performed within 6 days of the examination.

All the operations were performed on out patients by the same doctor who was an experienced gynaecologist. In all cases the procedure was dilatation by Hegar's method and vacuum aspiration under paracervical blockade. In addition the patients received diazepam 10 mg i.v.

After disinfection of the external genitalia vagina and cervix the cervix was grasped with single toothed tenaculum. The local anaesthetic for paracervical blockade (1% lidocaine-0.0005% adrenaline 10+10 ml) was injected in the usual way. The operation was started 5 min after the injection.

The uterus was sounded and dilated using Hegar's dilators. If the uterine cavity was less than 12 cm deep the cervix was dilated to Hegar No. 9. If the cavity was deeper the cervix was dilated to Hegar No. 11. The corresponding suction cannula was inserted into the uterine cavity. By rotating the cannula and drawing it downwards the contents of the uterine cavity were evacuated. Afterwards the cavity was explored with a

NUMB OF ABORTIONS

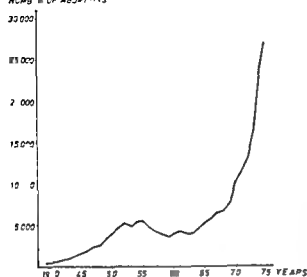


Fig 1 Annual number of legal abortions in Denmark during the last 35 years

small curette. All tissue removed was placed in formalin for subsequent histological examination. After the operation the patient was given methyl ergonovine maleate (Methergine®) 0.2 mg i.m. All Rhesus negative patients received anti D immunoglobulin i.m. This procedure has previously been described in detail by others (7, 6, 10).

The patient was then moved into a bed in a resting recovery ward where she was observed for 1–2 hours. Her vital signs were received during this period and she was checked for bleeding. If the patient's condition was satisfactory at the end of this period she left the hospital and was recommended to use no vaginal tampons and no coitus for 4 weeks after the abortion. She was also to see her doctor if fever, abdominal pain or severe bleeding developed. The patient was advised to come for a check up examination 1 week later by the doctor who had performed the operation. The women were examined with special reference to bleeding and infection of the uterus and uterine tubes.

Table I Age distribution of 1391 women applying for abortion

Age (y)	Patients	
	No	%
Under 16	12	0.9
16–20	225	16.1
21–25	485	34.8
26–30	354	25.4
31–35	169	12.1
36–40	109	7.8
41–45	30	2.2
Over 45	7	0.5
Total	1391	99.8

Table II Duration of pregnancy in 1391 applying for abortion

Duration of pregnancy (weeks)	Patients	
	No	%
Under 9	783	56.3
9–10	407	28.9
11–12	185	13.3
Over 12	21	1.5
Total	1391	100.0

At this one week check up examination all were asked whether they found that the surgical cure had given rise to considerable some or only discomfort and whether they would have oral anaesthesia.

RESULTS

The age distribution of the 1391 women is shown in Table I. The average age of the women applying for abortion was 26.3 years and 51.8% of them were years or under. The duration of pregnancy appears from Table II. Of the patients 783 (56.3%) were seen in or before the 8th week of pregnancy, 115 patients (1.5%) who were in the 13th week or more were refused abortion. Table III shows the parity of the women applying for termination of pregnancy: 760 (54.3%) were primigravidae and 49 (3.5%) had given birth to 4 or more children. Of the patients studied 242 (17.4%) had previously had 1 abortion — 8 of them (0.6%) on three or more (Table IV). A total of 1271 women (94.2%) turned for check up examination.

Among the 1349 patients 14 (i.e. 1.0%) had some complications associated with the abortion.

Table III Parity in 1391 women applying for abortion

Parity	Patients	
	No	%
0	760	54.3
1	232	16.7
2	249	17.9
3	101	7.3
4	34	2.4
Over 4	15	1.1
Total	1391	99.7

Table IV Number of previous abortions in 1391 women applying for abortion

No. of previous abortions	Patients	
	No	%
1	149	82.6
2	203	14.6
3	31	2.2
4	7	0.5
5	1	0.1
Total	1391	100.0

A rise of temperature to 38°C or more on 2 successive days after the day of operation or clinical signs of endometritis, salpingitis and parametritis occurred in 9 patients. In these cases the infections were treated conservatively with antibiotics and immobilization. Six of these patients were admitted to hospital. One had profuse bleeding requiring blood transfusion after the abortion. The bleeding subsided within a few hours and the patient was discharged 2 days later. Incomplete abortion necessitating repeat curettage was seen in 4 cases. No uterine perforations or cervical ruptures were encountered.

On account of the moderate incidence of complications we found it irrelevant to correlate them with the age of the patients or the duration of pregnancy. Among the patients 845 (i.e. 62.6%) experienced only slight discomfort in association with the operation while 107 (7.9%) complained of considerable discomfort. 65 of the patients (4.8%) would have required general anaesthesia (Table V).

Nearly all the abortions were performed before the result of the gonorrhoea reaction was obtained and it subsequently appeared that 18 women had had a therapeutic abortion in presence of untreated gonorrhoea. No postoperative complications occurred in these patients; in particular no signs of salpingitis developed.

DISCUSSION

Several authors have previously described a variety of techniques for the termination of pregnancy in the first trimester and have reported widely varying rates of complications (8, 11, 13, 16). The method used in this study, i.e. vacuum aspiration, is a well known procedure which has been reported to be safe and well suited for out patient intervention

(10, 14). The complication rates in such studies range from only a few to approx. 30% (7, 11). In the present study the overall complication rate was low, only 1%. The most serious complication encountered was a case of profuse bleeding which required blood transfusion. Repeat curettage had to be performed in 4 cases. A total of 11 patients (i.e. 0.8%) had to be admitted to hospital after the operation.

There are probably several reasons for the low complication rate in this study. It seems reasonable to assume that wide experience in this particular field may have contributed to this result. All the operations were performed by the same doctor who was an experienced specialist in gynaecology and who had performed a large number of terminations of pregnancy also before the period under consideration.

It is well established (5, 13) that the incidence of complications of therapeutic abortions is lowest in the 6th to 8th week of pregnancy and that it increases with increasing gestational age. In this study more than half of the patients were subjected to termination of pregnancy prior to the 8th week of pregnancy. This is considerably earlier than in other reports (13, 16).

All the abortions were performed under local anaesthesia (1% lidocaine-adrenaline). Whether this has influenced the degree of complications is unknown, but some reports (1, 7, 9) indicate that the blood loss in relation to operation is smaller when local anaesthesia is used.

It is remarkable that 18 patients were infected with *Neisseria gonorrhoea* in the lower genital tract when the abortion was performed. Postoperatively none of these patients showed signs of infections of the uterus or salpinges. In acute salpingitis N

Table V Degree of discomfort experienced by 1349 patients subjected to abortion

	Patients	
	No	%
Considerable discomfort	107	7.9
Some discomfort	319	23.6
Only slight discomfort	845	62.6
Unknown	78	5.7
Total	1349	99.8
Would have preferred general anaesthesia	65	4.8

gonorrhoea has been considered the most important causative agent (3 12 15). During recent years other bacteria mycoplasmas (especially *M. hominis*) and chlamydia have been associated with acute salpingitis (4 17) and it now seems that in less than half of the cases *N. gonorrhoea* is responsible for infections of the uterus or tubes (17).

We conclude that therapeutic abortions can be performed safely and with an acceptably low incidence of complications in out patients provided the operations are performed by an experienced gynaecologist. In this way the demand for hospital facilities arising from the introduction of free abortion is greatly reduced. The method does not seem to carry with it any great discomfort to the patients.

REFERENCES

1. Benic B M & Kupresanin M. Vacuum aspiration using pericervical block for legal abortion as an out patient procedure up to the 17th week of pregnancy. *Lancet* 2: 619 1971.
2. Buckle A E R, Anderson M & Loug K C. Vacuum aspiration of the uterus in therapeutic abortion. *Br Med J* 2: 456 1970.
3. Eschenbach D A & Holmes K K. Acute pelvic inflammatory disease. Current concepts of pathogenesis, etiology and management. *Clin Obstet Gynecol* 18: 35 1975.
4. Eschenbach D A, Buchanan T M, Pollock H M, Forsyth P S, Alexander E R, Lin J S, Wang S P, Wentworth B B, McCormack W M & Holmes K K. Polymicrobial etiology of acute pelvic inflammatory disease. *N Engl J Med* 293: 166 1975.
5. R. Polikliniska vakuumaspirationer. Läkartidningen 69: 4665 1972 (Swedish text).

6. Kerslake D & Casey D. Abortion induced by means of the uterine aspirator. *Obstet Gynecol* 38: 1967.
7. Moberg P, Sjöberg H & Wqvist N. The hazards of vacuum aspiration in late first trimester. *Acta Obstet Gynecol Scand* 54: 113 1975.
8. Møller B R, Diederich P, Hansen J T & Ørskov V. Legal termination of pregnancy. *Ugeskr Læger* 138: 329 1976 (Danish text).
9. Møller B R, Hansen J T & Mommensen S. To be published.
10. Nathanson B N. Suction curettage for early abortion. Experience with 645 cases. *Clin Obstet Gynecol* 14: 99 1971.
11. —. Ambulatory abortion. Experience with 3 cases. *New Engl J Med* 286: 403 1972.
12. Rees E & Annelis E H. Gonococcal salpingitis. *J Vener Dis* 45: 205 1969.
13. Stewart G K & Goldenstein P. Medical complications of therapeutic abortions. *Obstet Gynecol* 40: 539 1972.
14. Strausz I K & Schulman H. 400 abortions performed under local anesthesia. *Obstet Gynecol* 38: 199 1971.
15. Studdiford W E, Caspar W A & Scadron E. The persistence of gonococcal infection in the adnexa. *Surg Gynecol Obstet* 67: 176 1971.
16. Tietze C & Lewit S. Joint program on abortion (JPFA). Early medical complications of abortion. *Studies in Family Planning* 3: 97 1972.
17. Weström L. Diagnosis Aetiology Acute Salpingitis. Studentlitteratur, Lund 1976.

Submitted for publication Nov 3 1976

Birger R Møller
Miltonsvej 11
DK-8270 Højbjerg
Denmark

CYCLIC AND STEROID INDUCED CHANGES IN ADRENERGIC NEUROTRANSMITTER LEVEL OF GUINEA PIG UTERUS

G Thorbert P Alm and E Rosengren

*From the Departments of Obstetrics and Gynecology Histology and Pharmacology
Lund University Lund Sweden*

Abstract The uterine neurotransmitter noradrenaline was examined histochemically and fluorometrically in guinea pigs during the estrous cycle and after pretreatment with sex steroids. In diestrous animals noradrenaline levels were higher than in the estrous state. Treatment with estradiol or progesterone alone did not markedly influence the level of uterine adrenergic transmitter. However, areas combined estradiol and progesterone administration caused a clear-cut reduction in uterine noradrenaline. The quantitative changes in transmitter level did not reflect the marked alterations in weight during the various menstrual conditions and even inverse noradrenaline-weight relationships were observed. The findings offer further support for the concept of a steroid mediated influence on uterine neurotransmission where the uterine adrenergic nerves constitute a separate target for endocrine control, distinct from the rest of the uterus.

The guinea pig uterus receives a well developed supply of adrenergic nerves which innervate the myometrial smooth musculature as well as the uterine vascular bed (20-21). It has recently been demonstrated that neuronal noradrenaline in the uterus shows marked fluctuations during pregnancy, that for example the uterus is almost devoid of noradrenaline containing nerves at term (17). Since these dramatic effects on the uterine adrenergic nerves can be revealed also in horns lacking fetuses during unilateral pregnancy (20) it has been concluded that not only mechanical but also humoral factors are involved.

It is known that two kinds of adrenergic nerves which are anatomically and functionally different contribute to the innervation of the uterus (17-19) as a basis for understanding the process governing special functional properties of the uterine innervation (16) the anatomy of the adrenergic nerve stem supplying the guinea pig uterus has recently

been explored in detail (21) in order to elucidate a hormonal influence on the guinea pig uterine adrenergic nerves changes in the level of neuronal noradrenaline were followed during the normal estrous cycle and after the administration of sex steroids.

MATERIALS AND METHODS

Animals The study comprises a total of 31 virgin guinea pigs (body weight 400-600 g) of a mixed strain purchased from a local breeder. They were given a standard pellet diet (Astra Ewos Sweden) and water *ad lib*. The animals were maintained under constant conditions regarding illumination, temperature and air humidity. The estrous cycle was determined by daily inspection of the vagina and when open a smear was taken. Before the animals were included in the study at least one estrous cycle of normal length (15-18 days) including two complete estrous phases was observed. Animals having pronounced cornification on a predicted day were considered to be in the estrous phase. The day of maximal cornification before the appearance of leucocytes in the smear was designated day 1 of the cycle (7) and diestrous animals were killed on day 9 to day 11. From the diestrous animals a smear was also taken on the day when the animal was sacrificed and only animals with a clear leucocytic dominance were accepted for further study.

Histochemical and chemical procedures Adrenergic nerves were visualized histochemically using the fluorescence microscopic technique of Falck & Hillarp (3). Noradrenaline was determined chemically according to the method of Bertler et al. (2) as modified by Haggendal (10). The heart and the whole uterus (cervix and the two uterine horns) were used in each determination. A minute disc shaped piece was taken from the mid portion of one uterine horn and processed for the Falck-Hillarp histo-fluorescence technique (3).

Drugs Progesterone (ACO Sweden) and 17 β -estradiol (Draco Sweden) diluted in peanut oil were used. Six animals received 0.5 μ g 17 β -estradiol sc daily for 14 days. 4 animals received 2 mg progesterone sc daily for 7 days.

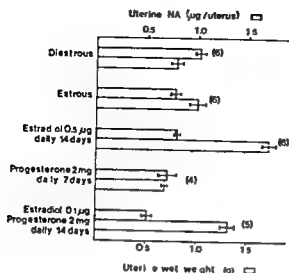


Fig 1 Fluorometric determinations of uterine noradrenaline (NA) and changes in organ weight during estrous cycle and after various sex steroid pretreatments. Mean \pm SE, number of determinations (=number of animals) within parenthesis.

and finally 9 animals were each given a combination of 0.1 µg 17 β -estradiol and 2 mg progesterone administered as two separate daily sc injections for 14 days. The only criterion for the selection of animals to be subjected to these steroid treatments was that the vagina was closed for three days before the start of the treatment.

Statistics. Student's *t* test was used in the evaluation of observed differences between mean values.

RESULTS

Strous cycle. The uterine adrenergic nerves did not display any clear differences in fluorescence intensity or in number when comparing estrous and diestrous animals. However, the chemical determinations demonstrated a somewhat higher total uterine noradrenaline content in the diestrous group compared to the estrous group ($p < 0.05$, Fig 1). On the other hand, the difference between the uterine noradrenaline concentrations (µg/g wet weight) was even more marked: 1.32 ± 0.11 µg/g (mean \pm SE) in diestrous and 0.80 ± 0.09 µg/g in estrous animals ($p < 0.01$).

Hormone treatment. Injections with either estradiol or progesterone did not produce any clear cut fluorescence microscopic change. Nor did the total content of uterine noradrenaline in the respective groups fluctuate significantly from estrous values ($p > 0.05$). When comparing these two groups to the group of diestrous animals, both

estradiol and progesterone treatment were associated with significantly lower uterine noradrenaline ($p < 0.01$ and $p < 0.05$ respectively, Fig 1).

Combined estradiol and progesterone treatment caused a moderate decrease in intensity of fluorescence and the number of fluorescent nerve terminals. This reduction was seemingly more pronounced in the main part of the uterine horn and less evident in the cervix and the tubal end of the uterine horn. This was accompanied by a decrease in the total uterine noradrenaline content when comparing either to the diestrous group ($p < 0.001$) or the estrous group ($p < 0.01$).

Uterine weights varied with the endocrine state (Fig 1). In diestrous animals the uterus was slightly lighter than in estrous animals though the difference was not statistically significant ($p > 0.05$). Both estradiol and combined estradiol/progesterone treatment caused statistically significant increase in uterine weight irrespective of whether the comparison was with the estrous or diestrous animals (estrus-treated animals *versus* estrous or diestrous, $p < 0.001$; estradiol and progesterone-treated animals *versus* diestrous group, $p < 0.001$ and *versus* estrous group, $p < 0.05$).

The noradrenaline content and weight of heart innervated by the classical type of sympathetic nerves did not fluctuate significantly when comparing the various experimental groups with each other ($p > 0.05$).

DISCUSSION

The guinea pig uterus contains considerable amounts of noradrenaline—but no measurable dopamine or adrenaline (19)—which is stored in the well-developed system of adrenergic nerves within the organ (20, 21). These adrenergic nerves originate partly from pre- and paravertebral ganglia and partly from paracervical ganglion formation (21) which give rise to the so-called short adrenergic nerves (17). There is a considerable regional variation in the density of the plexus of nerve terminals belonging to short adrenergic nerves as well as classical adrenergic nerves, both of which supply the myometrial and vascular smooth muscles in the organ. In the rabbit the uterine adrenergic nerves have a different organization in that only short adrenergic nerves supply the myometrium whereas the classical type innervates the vascular bed and no regional variation is

supply has been demonstrated. The system of port adrenergic neurons is in many respects functionally different from sympathetic nerves outside the urogenital tract; for example, they can be influenced by treatment with sex steroids, which can be revealed in terms of alterations in the amount of the noradrenaline transmitter (17-19).

Substantial fluctuations in the noradrenaline level in relation to the estrous and diestrous phase could be demonstrated in the guinea pig uterus, which suggests that the adrenergic nerves are also influenced by the cyclic hormonal changes occurring under physiological circumstances. This is supported by corresponding cyclic changes in the noradrenaline transmitter of human and monkey uterine products (8-15), in which the high level during the estral phase agrees well with the values for guinea pig uterus during diestrous. This comparison is particularly relevant since the guinea pig estrous cycle has many similarities to the primate menstrual cycle, including changes in sex steroids (7-11). It is interesting to note that endogenous fluctuations in uterine noradrenaline have also been found during reproductive cycles associated with seasonal variations in the hedgehog (13). It has been claimed in some studies on rats that cyclic fluctuations occur in uterine adrenaline. However, there is no evidence that adrenaline has a neuronal localization in the uterus, and it is therefore conceivable that any cyclic changes involve extraneuronal stores of this amine (14).

Treatment of guinea pigs with exogenous estrogen gave similar values for uterine noradrenaline as those measured during the estrous phase, although the hormone treatment caused a more conspicuous increase in the organ weight. Administration of progesterone alone caused a less pronounced reduction in noradrenaline than did treatment in combination with estrogen (9).

The dose of progesterone and estradiol used in the combined treatment group was selected from the work of Challis et al. (5) in order to produce an oestrogen state with similarities to that of pregnancy. In the guinea pig (10) as well as in the rabbit (18) and humans (12), pregnancy causes a prominent increase in uterine noradrenaline. That sex steroids contribute to this decrease is suggested by the reduction of noradrenaline seen in the guinea pigs receiving progesterone together with estrogen. This is further supported by the finding of a decrease in uterine noradrenaline transmitter in rab-

bits during HCG induced pseudopregnancy under conditions when both plasma and uterine progesterone are elevated (1-22).

Alterations in uterine noradrenaline in the different experimental groups did not parallel the changes in organ weight. Thus, opposite effects on weight and noradrenaline were observed during the estrous cycle as well as after the combined estrogen and progesterone treatment; a prominent weight gain with no change in noradrenaline was seen after the estrogen stimulation. This means that the normal fluctuations in uterine noradrenaline concentration expressed on an organ weight basis are quite considerable and therefore that the exact cycle stage has to be determined when material from guinea pigs is used as experimental control. There is thus further evidence that the uterine adrenergic nerves constitute a target system for steroid hormones separate from the organ proper as reflected by its weight. A similar conclusion was drawn from an entirely different experimental model in which the development of the genital tract and its adrenergic innervation was followed after interference of the early differentiation of the hypothalamus by postnatal castration or androgenization of male and female rats, respectively (4).

REFERENCES

1. Alm P, Falck B, Owman Ch, Sjöberg N-O & Thorbert G. Reduced level of uterine norepinephrine transmitter during hCG induced pseudopregnancy in the rabbit. *Endocrinology* 96: 819, 1975.
2. Bertler Å, Carlsson A, Rosengren E & Waldeck B. A method for the fluorimetric determination of adrenaline, noradrenaline and dopamine in tissues. *Kungl Fysiogr Sällsk Lund Forh* 28: 191, 1958.
3. Björklund A, Falck B & Owman Ch. Fluorescence microscopic and microspectrofluorimetric techniques for the cellular localization and characterization of biogenic amines. In: *Methods of Investigative and Diagnostic Endocrinology* (ed S A Berson). The Thyroid and Biogenic Amines (ed J E Rall & I J Kopin), p. 318. North Holland Publication Company, Amsterdam, 1972.
4. Broberg A, Nybøll G, Owman Ch, Rosengren E & Sjöberg N-O. Consequence of neonatal androgenization and castration for future levels of norepinephrine transmitter in uterus and vas deferens of the rat. *Neuroendocrinology* 15: 308, 1974.
5. Challis J R G, Heap R B & Illingworth D V. Concentrations of oestrogen and progesterone in the plasma of non-pregnant, pregnant and lactating guinea pigs. *J Endocr* 51: 333, 1971.
6. Croix M & Franchimont M. Changes in the serum levels of the gonadotrophins, progesterone and

- estradiol during the estrous cycle of the guinea pig *Neuroendocrinology* 19 1 1975
- 7 Donovan H T & Lockhart A N Growth and regression of the corpora lutea formed in guinea pigs in response to treatment with exogenous gonadotrophin *J Endocrinol* 54 377 1972
 - 8 Dujoune A R de Laborde N P Camil L M Cheviakoff B Pedroza E & Rosner J M Correlation between catecholamine content of the human Fallopian tube and the uterus and plasma levels of estradiol and progesterone *Am J Obstet Gynecol* 124 229 1976
 - 9 Falck B Owman Ch Rosengren E & Sjöberg N O Reduction by progesterone of the estrogen induced increase in transmitter level of the short adrenergic neurons innervating the uterus *Endocrinology* 84 958 1969
 - 10 Hägglund J An improved method for fluorometric determination of small amounts of adrenaline and noradrenaline in plasma and tissues *Acta Physiol Scand* 59 242 1963
 - 11 Hilliard J Corpus luteum function in guinea pigs hamsters rats mice and rabbits *Biol Repr* 6 203 1973
 - 12 Nakanishi H McLean J Wood C & Burnstock G The role of sympathetic nerves in control of the nonpregnant and pregnant human uterus *J Reprod Med* 9 20 1969
 - 13 Nielsen C & Owman Ch Sympathetic nervous system II Histochemistry *In* Seasonal Variations in the Physiology and Biochemistry of the European Hedgehog (*Erinaceus europaeus*) Including Comparisons with Non Hibernators Guinea Pig and Man (ed H W Johansson & J B Senturia) *Acta Physiol Scand Suppl* 380 1 1972
 - 14 Owman Ch Alm P Hokfelt T Rosengren E Sjöberg N O Swedin G & Thorbert G Catecholamines in rat uterus and paracervical ganglion A histochemical chemical and microspectrofluorometric study *In* manuscript
 - 15 Owman Ch Falck B Johansson E B Rosengren E Sjöberg N O Spörting B Svensson K-G & Wallis J Autonomic nerves and related amine receptors mediating motor activity in the oviduct of monkey and man A histochemical chemical and pharmacological study *In* Ovum Transport and Fertility Regulation (ed M J K Harper C Pauerstein C E Adams M Coutinho R Crovatto & M Paton) p 256 Scand Copenhagen 1976
 - 16 Owman Ch & Sjöberg N O Effect of progesterone and sex hormones on the transmitter level in short adrenergic neurons *In* Frontiers in Catecholamine Research (ed E Usdin & S Snyder) p 7 Pergamon Press Oxford 1973
 - 17 Owman Ch Sjöberg N O & Spörting B Short adrenergic neurons a peripheral amine mechanism *In* Fluorescence Histochemistry Biogenic Amines (ed M Fujiwara) p 47 Shoin Tokyo 1974
 - 18 Rosengren E & Sjöberg N O Changes in amount of adrenergic transmitter in the tract of rabbit during pregnancy *Acta Physiol* 72 412 1968
 - 19 Sjöberg N O The adrenergic transmitter of female reproductive tract Distribution changes *Acta Physiol Scand Suppl* 305 1 1967
 - 20 Sjöberg N O Considerations on the cause of disappearance of the adrenergic transmitter in the nerves during pregnancy *Acta Physiol Scand* 7 1968
 - 21 Thorbert G Alm P Owman Ch & Sjöberg N O Regional distribution of autonomic innervation in guinea pig uterus *Am J Physiol* 233 C25 1977
 - 22 Thorbert G Batra S Owman Ch Rosengren E & Sjöberg N O Uterine norepinephrine levels related to plasma and tissue progesterone in pregnant rabbits *Endocrinology* 99 1707 1976

Submitted for publication Feb 27 1977

Gunnar Thorbert
Department of Obstetrics and Gynecology
University Hospital
S 22185 Lund
Sweden

URETHRAL PRESSURE PROFILE BEFORE DURING AND AFTER PUBOCOCCYGEAL REPAIR FOR STRESS INCONTINENCE

A Öbrink G Bunne U Ulmsten and
A Ingelman Sundberg

*From the Department of Obstetrics and Gynecology Karolinska Institutet
Sabbatsberg Hospital Stockholm Sweden*

Abstract In two groups of women the urethral pressure profile was recorded using a microtransducer catheter. The two groups were of approximately the same age, one consisting of continent women, the other of women with stress incontinence. Sixteen stress incontinent women were examined before and after pubococcygeal repair and 10 of these were also examined during the operation. The two groups were compared for differences in the urethral pressure profile in the continent and incontinent states. The stress incontinent women had a significantly shorter functional length of the urethra (10 mm) but the same maximal urethral pressure as continent women. No remarkable changes in these parameters were seen during the operation. However, at the examination 3 months postoperatively they had totally disappeared. The only change in the urethral pressure profile which persisted after the operation was a higher pressure in the proximal part of the urethra so that a urethral maximal pressure plateau had been established. This plateau was also seen at a higher level in the continent state.

Stress incontinence is characterized by urinary leakage at a sudden intra abdominal pressure increase. Why a woman becomes continent after a successful operation is still only partly understood. Presumably it has been supposed to be connected with the bladder neck being elevated from its pathologically lowered position.

The significance of the urethra's length for the maintenance of continence has been debated. Pridmore (7) reported that an abnormally short urethra could explain the existence of stress incontinence. This hypothesis was based on measurements made with a Foley catheter inside the urethra in erect position and the application of the law of Laplace to the urethra.

Wlodjkinson (4) using the bead chain technique

did not find a shorter anatomical length of the urethra in stress incontinent women than in continent ones.

In 1961 Enhörning (3) showed that neither the anatomical nor the physiological length of the urethra was different in stress incontinent women compared with continent ones (the physiological length according to Enhörning is the distance from the point proximally where the urethral pressure begins to rise above the bladder pressure to the external urethral meatus). The maximal pressure of the urethra was shown to be significantly lower in cases of stress incontinence compared with that in continent women. The measurements were performed with simultaneous urethrocystometry using a balloon catheter connected to conventional transducers of variable inductance type. The equipment was technically complicated and did not allow continuous recording of the urethral pressure profile simultaneously with the cystometry.

A new technique for pressure recordings has been reported (9). It is based on the use of a thin catheter with two microtransducers inside (Millar Instrument Inc, Houston, USA) connected to conventional amplifiers and to a recorder. This method of pressure recording has good precision and reproducibility (1, 2). Hence it was chosen for this investigation.

The main purpose of the investigation was to study whether any specific changes in pressures in the urethra and bladder were correlated to the transformation from incontinence to continence at surgical treatment. Therefore the functional length of the urethra, the maximal urethral pressure, the

bladder pressure and the urethral closure pressure in stress incontinent women were compared before and after operation. Furthermore the urethral pressure profile was recorded at different steps of the pubococcygeal repair operation. This we did in order to get a focused picture of the changes in the parameters when the patient became continent. A comparison was also made between a control group of continent women and the stress incontinent women pre and postoperatively.

MATERIAL

A group of 16 stress incontinent women and a control group of 14 continent women were examined.

The stress incontinent group

Sixteen women with a mean age of 57 years ($r=77-31$) and a mean parity of 2.3 ($r=4-0$) with genuine stress incontinence were examined. All patients suffered from at least stress incontinence of degree II on a scale of three (6) (I) urinary leakage only at coughing or sneezing (II) urinary leakage also at moderate strain (e.g. walking quickly) (III) urinary leakage even at minimal strain (e.g. changing position).

None of the patients had previously been operated on either abdominally or vaginally on any gynecological indication. Preoperatively the patients had been subject to a uro-gynecological examination including a neurological status of the dermatomes S1-S4, urethrocytostomy, cystometry (Lewis) and sphincterometry (Leander). In all patients a downward rotation of the anterior vaginal wall was seen at coughing.

Leakage was present at coughing at a bladder volume of 200 ml and Bonney's test was positive. Urinary cultures were negative. All patients had practiced holding urine for at least half a year and had preoperatively been with estrogens (Promant[®], Progynon[®] p.o. or 10-20 mg i.m.). Ten women in this group chosen at random were also examined during the operation. All 16 women were examined 3 months after the pubococcygeal repair operation.

The control group

Fourteen volunteering continent women with a mean age of 54 years ($r=64-48$) and a mean parity of 1.2 ($r=3-1$) were examined. None of the patients suffered from incontinence or other disorders of the urinary tract. Urinary cultures were negative and the gynecological findings were normal. All women were informed of the aim of the investigation and gave their verbal consent to participation.

METHOD

Simultaneous urethrocytometry with the urethral pressure profile was performed (9). The recording equipment consisted of a semiflexible dacron catheter with two microtransducers enclosed (outer diameter 2.31 mm), three amplifiers and an ink jet recorder (Siemens Elema, Stockholm, Sweden). The pressure sensitive areas (diameter

0.75 mm²) were situated one at the tip of the other 60 mm proximally. These areas were connected to the microtransducers in the catheter according to strain gauge principle. This pressure recording technique has been described (17) and found to have good precision and reproducibility. With this equipment the bladder pressure, the urethral pressure and the closure pressure were determined simultaneously. To record the urethral pressure profile and thereby the functional length of urethra we used a synchronous motor with an arm catheter could be attached and withdrawn from the bladder with a velocity of 2.5 mm/s.

In both groups of patients three urethral pressure files at rest were recorded at every 100 ml increase from 100 ml until the maximal bladder capacity was reached. Simultaneously the bladder pressure was recorded. The holding urine profiles (bladder volume of 200 ml). The stress incontinent women were examined preoperatively and three months postoperatively.

During the operation the urethral pressure profile was recorded after every step of the operation at a constant bladder volume of 300 ml 0.9% saline solution (checked three times during the operation). Seven patients among those examined preoperatively were also examined two weeks after surgery. This was done with a bladder volume of 300 ml or 200 ml if the patient reported stress incontinence at this bladder volume.

Operation method ad modum Ingelman Sundberg

The steps during pubococcygeal repair are:

I Anaesthesia (epidural in all cases except one).
II An arcuate incision is made below the external urethral meatus. The anterior vaginal wall is dissected from the urethra and from the bladder floor up to the cervix.

III The bladder ligaments are dissected and sutured along the midline from the proximal urethra to the cervix. The innermost suture is fixed. Continence is tested at 300 ml in the bladder at repeated coughs. If still incontinent at this stage, some more ligaments are added until the bladder neck region is sufficiently elevated and the patient continent.

IV The pubococcygeal muscles are dissected and totally divided just below the middle.

V The anterior portions of the pubococcygeal muscles are sutured under the proximal part of the urethra and the bladder neck, thus creating a muscle sling under the bladder ligaments.

VI The bulbocavernosus muscles are sutured along the midline without division as a support for the distal part of the urethra. The operation is finished by suturing the posterior portions of the pubococcygeal muscles to the ischio-cavernosus muscles bilaterally. The anterior wall is sutured at the introitus, a suprapubic tamponade applied as well as a vaginal tamponade.

Definitions and interpretations

Definitions according to the ICS Standard Committee (1975) (5).

Urethral pressure The pressure within the urethra recorded in relation to the atmospheric pressure.

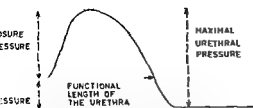


Fig 1 The curved line represents the recorded pressure in the bladder along the urethra to the external meatus

enecal pressure The pressure within the bladder recorded in relation to the atmospheric pressure

Urethral pressure profile Continuous recording of the intraluminal pressure throughout the entire length of the urethra

Urethral closure pressure The intraurethral pressure minus the bladder pressure

The functional length of the urethra The part of the urethra where the intraluminal pressure exceeds the bladder pressure

How the parameters are measured from the records is shown in figure 1 above

Three urethral pressure profiles were measured at every 100 ml bladder volume but only the third one was counted for the statistical analysis. The reason for this was that some women had more sensitive urethras than others. Voluntary movements and contractions of the pelvic floor might distort the urethral pressure profile. Repeated recordings of 10 consecutive profiles under equal conditions showed that the profiles were almost identical from the third one onwards.

RESULTS

Control group

The mean functional length of the urethra decreased from 32 (r=44-22.5) to 29 (r=42-20) mm when the bladder volume was increased from 100 to 400 ml ($p < 0.01$). The mean physiological length at 200 ml bladder volume was 37 mm. The mean distance between the end of the pressure sensor and the external urethral meatus was 15 mm.

The mean maximal urethral pressure did not change with increasing bladder volume. The mean values were approximately 38 mmHg. The urethral pressure was maximal along approximately 15 mm of the profile. In that part of the urethra where the pressure was highest there were pressure variations synchronous to the patient's breath and pulse. These amplitudes were 5-10 and 2-5 mmHg respectively. As the pressure sensor area of the cath-

eter moved along the urethra during measurement of the pressure profile these amplitudes diminished.

The mean bladder pressure increased from 8.5 mmHg (r=13-5) to 11 mmHg (r=15-7) ($p < 0.01$). A slow continuous pressure increase was seen in all patients.

The differences in the parameters at 100 and 400 ml bladder volume were analyzed with Student's *t* test.

Light tenesmus occurred at a mean bladder volume of 350 ml (r=550-200). Severe tenesmus occurred at a mean bladder volume of 600 ml (r=800-400). The mean total bladder capacity was 600 ml (r=800-550). The mean urinary residual volume was 4 ml (r=20-0).

The urethral pressure profile at holding urine was recorded in all patients. Only five patients could hold urine without a simultaneous contraction of the abdominal muscles. Changes in both the functional length and the maximal pressure of the urethra were seen when the profiles of these patients at rest and at holding urine were compared at the same bladder volume.

The stress incontinent group preoperatively

The mean functional length decreased from 23.5 (r=33-17) to 19.5 (r=28-13) mm when bladder volume was increased from 100 to 400 ml ($p < 0.01$). The mean physiological length at 200 ml bladder volume was 27.5 mm. The mean distance between the maximal pressure point and the external urethral meatus was 12 mm.

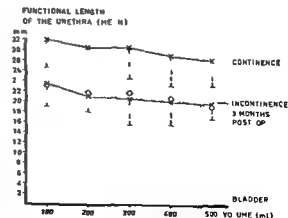


Fig 2 The change in the functional length of the urethra at increasing bladder filling in continent and incontinent women

The mean maximal urethral pressure did not change with increasing bladder volume. The mean values were approximately 38 mmHg. The urethral pressure profile had a pointed appearance with a distinct maximal point. At the point in the urethra where the pressure was highest there were pressure variations synchronous to the patient's breath and pulse. Their amplitudes were 5–10 and 2–5 mmHg respectively.

The mean bladder pressure increased from 12.4 ($r=21-7$) to 14 ($r=24-9$) mmHg ($p<0.01$). A slow continuous pressure increase was seen in all patients.

The differences in the parameters at 100 and 400 ml bladder volume were analyzed with Student's *t* test.

The mean values at 300 ml bladder volume for the 10 patients who were also recorded during the operation were

$F=21.5$ mm $U=41$ mmHg $B=14$ mmHg
($r=29-13$) ($r=60-28$) ($r=33-8$)

F = mean functional length of the urethra

U = mean maximal urethral pressure

B = mean bladder pressure

Light tenesmi occurred at a mean bladder volume of 225 ml ($r=300-100$). Severe tenesmi occurred at a mean bladder volume of 400 ml ($r=500-300$).

The mean total bladder capacity was 450 ml ($r=500-300$). The mean urinary residual volume was 7 ml ($r=20-0$).

The urethral pressure profile at holding urine was recorded in all patients. Only 5 patients could hold urine without a simultaneous contraction of the abdominal muscles. Changes in both the functional length and the maximal pressure of the urethra were seen when the profiles of these patients were compared at rest and at holding urine at the same bladder volume.

The stress incontinent group during the operation

The mean functional length increased from 19.5 mm ($r=23-16.5$) after anaesthesia (step I) to 37.5 mm ($r=44-29$) after the bulbocavernous suture (step VI). Some increase was seen after the dissection but the largest increase occurred after suture of the bladder ligaments and the bulbocavernous muscles.

The mean maximal urethral pressure increased from 26 ($r=44-13.5$) to 31.5 ($r=48-12.5$) mmHg. The total increase occurred after the bladder ligaments had been sutured.

The mean bladder pressure decreased in spite of a constant bladder volume from 10 ($r=17-4.5$) to 8 ($r=10-2.5$) mmHg ($p<0.01$).

The stress incontinent group 2 weeks postoperatively

$F=30$ mm $U=35.5$ mmHg $B=13$ mmHg

Because several patients still had their suprapubic catheters and some of them had urinary infection, calculations regarding the urethral pressure values must be made with caution.

The stress incontinent group 3 months postoperatively

Fifteen of the 16 stress incontinent women were cured and one improved at the examination 3 months postoperatively. The bladder neck well elevated and no downward rotation of the anterior vaginal wall was seen at strain in the patient.

The mean functional length increased from 18.5 ($r=28.5-16$) to 20.5 ($r=25.5-10$) mm when bladder volume was increased from 100 to 400 ml ($p<0.05$).

The mean maximal urethral pressure did not change with increasing bladder volume. The mean values were approximately 32 mmHg. At the point of the urethra where the pressure was highest there were pressure variations synchronous to the patient's breath and pulse. Their amplitudes were 2–5 mmHg respectively. The urethral pressure profile had a broad maximal plateau.

The mean bladder pressure increased from 8 ($r=20-7$) to 15.5 ($r=26-9$) mmHg ($p<0.01$). A slow continuous pressure increase was seen in all patients.

The differences in the parameters at 100 and 400 ml bladder volume were analyzed with Student's *t* test.

The mean values at 300 ml bladder volume for the 10 patients who had also been recorded during the operation were

$F=22.5$ mm $U=36.5$ mmHg $B=15.5$ mmHg
($r=26.5-18$) ($r=45-23$) ($r=24-8.5$)

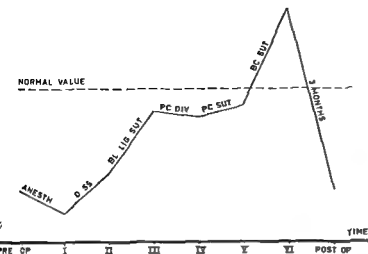
FUNCTIONAL
LENGTH OF THE URETHRA (MEAN)

Fig 3 Variations in the mean functional length of the urethra pre per and postoperatively compared to the normal value

ht tenesmi occurred at a mean bladder volume of 480 ml ($r=350-200$) Severe tenesmi occurred at a mean bladder volume of 480 ml ($r=600-350$) The mean total bladder capacity was 490 ml ($r=600-400$) The mean urinary residual volume was 20 ml ($r=90-0$)

The urethral pressure profile was recorded in all patients at holding urine Ten out of 16 patients could hold urine without a simultaneous contraction of the abdominal muscles Five of these patients did show changes in the urethral functional length and the maximal urethral pressure when comparisons were made with the profiles at rest at the same bladder volume

The urethral pressure profile at rest and at holding urine

		At rest	At holding urine
Functional length of the urethra (mm)	Preop	21.5	27 = 5.5
	Postop	22	25 = 3
	Normal	28	34 = 6
Maximal urethral pressure (mmHg)	Preop	41.5	50 = 8.5
	Postop	27.5	37 = 9.5
	Normal	47	56.5 = 9.5

All measurements were made at a bladder volume of 200 ml After the operation contraction of the muscle sling brings the urethra chiefly anteriorly without any lengthening

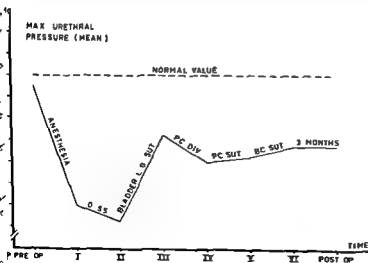


Fig 4 Variations in the mean maximal urethral pressure pre per and postoperatively compared to the normal value

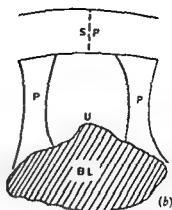
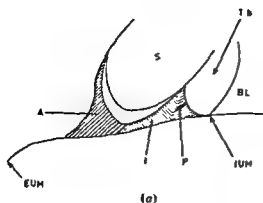


Fig 5 Schematic drawing of the urethral suspension (a) Sagittal view (b) As indicated by the arrow in (a) A=anterior pubourethral ligament BL=bladder EUM=

external urethral meatus IUM=internal urethral meatus I=intermediate pubourethral ligament P=posterior pubourethral ligament SP=symphysis pubis U=urethra

DISCUSSION

Stress incontinence versus continence

The prominent features of the parameters recorded preoperatively in the stress incontinent group were that the functional length of the urethra was nearly 10 mm shorter in the incontinent patients compared with the continent (see Fig 2). The urethral maximal pressure did not differ between the two groups.

The shortening of the functional length mainly affected the proximal part of the urethra. Is the short urethral functional length a consequence of the lost ability of some part of the urethra to maintain a closure pressure or does it have to do with a

loss of the true anatomical length or is it

rather a combination of the two? According to Hodgkinson (4) the anatomical length is the same in incontinent and continent conditions. If this is true the short functional length at incontinence depends on a loss of tone in the proximal part of the urethra secondary to the lowering when the fixation to the symphysis has deteriorated. This reduced tissue resistance renders the pressure in the proximal part (7 mm) of the urethra equal to the bladder pressure and this part of the urethra must consequently be filled with urine. Possibly this loss of tone gives a more or less prominent funnelling of the proximal urethra as has been shown in radiographic studies (8). However the bead chain technique used by Hodgkinson had a methodical error of ± 5 mm. The possibility of a minor shortening of the anatomical length caused by the lost symphyseal fixation is therefore not excluded but on the contrary highly probable. The urethra is closely attached to the

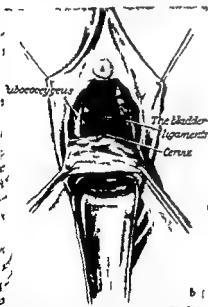
anterior vaginal wall so that when both structures lose their fixation they are not only separated but also shortened. Thus it seems that the short functional urethral length, the loss of tone, funnelling and perhaps a shortened anatomical length are consequences of the same cause, i.e. the lowered position of the upper urethra. Normally the bladder ligaments act as a hammock supporting the bladder neck. Furthermore, the proximal urethra is held in position and stretched by the fairly strong elastic posterior pubo-urethral ligaments containing collagen and elastic connective tissue (Fig 1). When the bladder ligaments lose their attachment to each other and glide apart the bladder neck loses its support from beneath and the strain on the pubo-urethral ligaments becomes too great. Consequently the urethra is only fixed to the symphysis by the anterior pubo-urethral ligament and by the intermediate ligaments which possibly relax gradually. Thus the whole anterior vaginal wall rotates downwards at straining. This means that all parts of the urethra can be shortened by the weight of the full bladder.

This theory is supported by the fact that the functional urethral length in stress incontinence is uniformly shortened at increasing bladder volume. In the control group the numerical shortening of the functional length was the same (Fig 7) but involved only the upper part of the urethra down to

Fig 6a-f Step wise illustrations of the operation and the corresponding urethral pressure profile in a stress incontinent woman

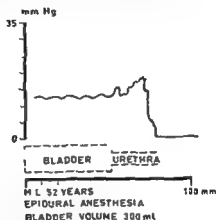


Step I Epidural anaesthesia reduced both the maximal urethral pressure and the bladder pressure by approximately 1/3 (41–6 mmHg and 14–10 mmHg respectively). The functional urethral length was unaffected. In all operations the dermatomes Th9–S4 were anaesthetized. Thus the sympathetic and the parasympathetic nerves to the



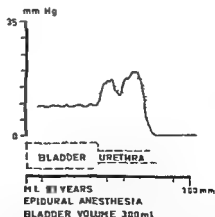
Step II At the detachment of the urethra from the vaginal wall the pressure in the proximal urethra increased markedly in almost all patients. In some way the vaginal wall seemed to have a diminishing effect on the pressure in the proximal urethra. As long as the urethra was attached to the vaginal wall the elastic tissue of the posterior

FUNCTIONAL LENGTH OF THE URETHRA 21 mm
AREA OF THE FUNCTIONAL URETHRAL
PRESSURE PROFILE 100 mm²
URETHRAL CLOSURE PRESSURE 7 mm Hg
BLADDER PRESSURE 12 mm Hg

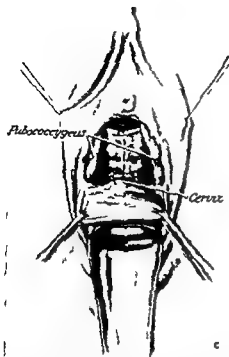


smooth muscles were blocked. The motor fibers to the striated muscles of the pelvic floor were at least partly blocked. Thus the remaining urethral pressure must be maintained by the smooth muscles themselves and to some degree by the striated muscles, not blocked.

FUNCTIONAL LENGTH OF THE URETHRA 29 mm
AREA OF THE FUNCTIONAL URETHRAL
PRESSURE PROFILE 370 mm²
URETHRAL CLOSURE PRESSURE 10 mm Hg
BLADDER PRESSURE 9 mm Hg

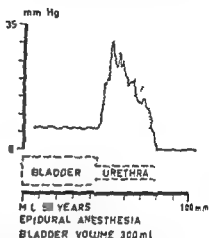


pubo-urethral ligaments could probably not influence the pressure. When the urethra was released perhaps these ligaments both elevated the position and increased the tone of the proximal urethra. The functional length was unaltered except in three patients where an increase was seen.



Step III Repair of the bladder ligaments elevated the bladder floor, the bladder neck and the urethra. The functional urethral length increased prominently and the maximal urethral pressure slightly. These changes were not correlated to each other, i.e. an increase in functional length did not bring about a proportional increase in maximal urethral pressure. Possibly these changes were entirely mechanical. The bladder ligaments were tightly sutured under the bladder neck and approximately 5 mm of the upper urethra. By sheer compression the pressure increased in that part of the urethra which in the inconti-

FUNCTIONAL LENGTH OF THE URETHRA 32 cm
AREA OF THE FUNCTIONAL URETHRAL
PRESSURE PROFILE 705 mm²
URETHRAL CLOSURE PRESSURE 28 mm Hg
BLADDER PRESSURE 6 mm Hg



nent condition physiologically belonged to the high. When the urethra was elevated the space between it and the symphysis diminished. Thereby the ad-pose tissue in this space was compressed and exerted a pressure on the whole length of the urethral wall. Since the urethra cannot yield to this pressure, not only the maximal pressure point, but the whole urethral pressure profile increased. After this phase of the operation there was a solid increase in pressure along the whole urethra; the closure pressure had increased 10 mmHg, the bladder neck was in the position, and the patients were continent.

where the posterior pubo-urethral ligaments are no longer effective (the point on the urethral pressure profile where the pressure starts declining).

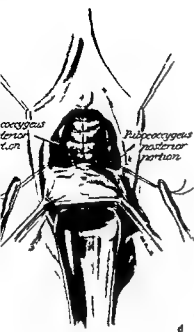
The maximal urethral pressure in the stress incontinent group was as high as in the continent control group and no changes occurred as the bladder was filled. However, a remarkable difference was that in most cases of stress incontinence the maximal pressure plateau was reduced to a maximal pressure point. According to Enhorning, stress incontinence is characterized by an abnormally low urethral pressure. The discrepancy between his results and ours could be explained partly by the fact that the patients in this investigation had not been operated on before. As will be discussed later on, an operation of this kind tends to lower the urethral pressure by way of either denervation or scarring, or both.

Stress incontinence during surgical treatment

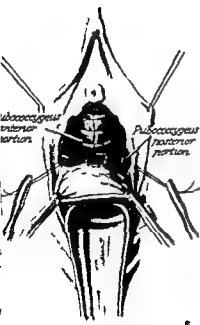
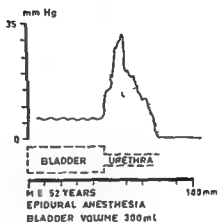
The pubococcygeal repair caused a doubling of urethral functional length and a slight increase in the maximal urethral pressure. The bladder pressure decreased by 1/3 (10-7 mmHg) in spite of constant bladder volume. Thereby the stress incontinence (closure pressure) increased. All steps in the operation brought about most of the changes (Table I and Fig. 6a-d).

Two weeks after surgical treatment

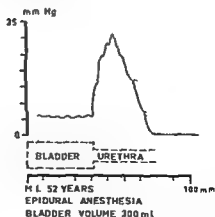
From the appearance of the urethral pressure profile 2 weeks postoperatively it was obvious that the effect of the bulbocavernosus muscle suture had appeared in most patients. Besides, some patients had a more pronounced shortening of the functional length, probably caused by a slackening of the sutured bladder ligaments.



FUNCTIONAL LENGTH OF THE URETHRA 30 mm
 AREA OF THE FUNCTIONAL URETHRAL
 PRESSURE PROFILE 705 mm²
 URETHRAL CLOSURE PRESSURE 24 mm Hg
 BLADDER PRESSURE 6 mm Hg

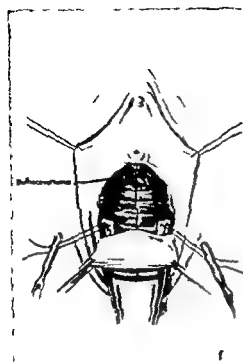


FUNCTIONAL LENGTH OF THE URETHRA 32 mm
 AREA OF THE FUNCTIONAL URETHRAL
 PRESSURE PROFILE 834 mm²
 URETHRAL CLOSURE PRESSURE 25 mm Hg
 BLADDER PRESSURE 8 mm Hg

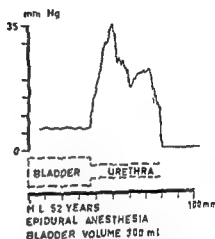


steps IV and V. When the pubococcygeal muscles had been divided and sutured no significant changes in the pressures were recorded. However the appearance of the pressure profile changed markedly so that the profile became more pointed. This implies that the levators were

not totally anaesthetized by the epidural anaesthesia. Another important conclusion was that the levators contribute to the urethral pressure at rest although these muscles have been shown to have no muscle fibers intermingled in the urethral wall.



FUNCTIONAL LENGTH OF THE URETHRA 43 mm
 AREA OF THE FUNCTIONAL URETHRAL
 PRESSURE PROFILE 1291 mm²
 URETHRAL CLOSURE PRESSURE 28 mm Hg
 BLADDER PRESSURE 6 mm Hg



Step VI The bulbocavernous suture brought about a sharp rise of the urethral pressure profile distally and thus an increase in the functional length. This must be due

entirely to the hard compression exerted by the urethral bulbocavernous muscles creating a sling under the distal part of the urethra.

The maximal urethral pressure was lower than would have been anticipated from the last recordings during the operation even allowing for the calculated influence of the anaesthesia. The reason for this decrease could probably be the diminished compressing effect of the bladder ligament and the relaxation at the urethral dissection.

Three months after surgical treatment

Of the 16 patients 15 were cured and 1 improved. The elevated position of the bladder neck and the

proximal urethra was maintained in all cases. The functional length of the urethra was reduced to the same level as before the operation. In most cases the lengthening effect of the bulbocavernous muscle suture had almost disappeared or the suture was located so far distally that it did not enhance the urethral functional length. In this reduction of the functional length there was a decrease probably caused by slackening of the bladder ligaments. Thereby the compression of the most proximal part of the urethra was lost and

Table I Summary of the parameters for the woman in Fig. 3

The urethral pressure profile during pubococcygeal repair of stress incontinence					
	Functional length (mm)	Functional area (mm ²)	Closure pressure	Bladder pressure	Max urethral pressure
Anaesthesia	21	100	7	12	19
Vaginal wall dissected	28	370	10	9	19
Bladder lig. sutured	33	706	26	6	37
Pubococcygeal muscles cut	30	705	24	6	30
Pubococcygeal muscles sutured	32	834	25	6	31
Bulbocavernous muscle sutured	43	1291	28	6	34

umed its original appearance 1 = physiologically ad become part of the bladder

From the operative recordings it might have been pected that the high urethral pressure would sist to some extent after the operation and give ncreased margin to incontinence. However, eady 2 weeks after the operation the urethral ssure had been reduced and after 3 months it s further reduced and even lower than in the ontinent condition before the operation. The thra was no longer compressed against the mphysis. In most patients there was a definite rease in the urethral pressure at the division of pubococcygeal muscles during the operation, hich did not always influence the maximal pres e. This could partly explain the urethral pressure duction. Probably a third contribution to the pres e decrease was denervation brought about at urethral and bladder dissection. The importance postoperative urethral scarring cannot be estiated with certainty after this short observation te.

The pointed appearance of the urethral pressure files before the operation was changed post eratively. Now most profiles had a broad pres e plateau similar to those seen in the control up but of significantly lower magnitude. Perhaps s pressure plateau is an advantage for the maintenance of continence. The pressure plateau indicates an increase in the pressure in the proximal rt of the urethra. This could be explained by a over of urethral tone accomplished when the thra is released from the vaginal wall and by er compression from the supporting tissues (the vic fascia and the pubococcygeal muscle sling) g 7).

Recordings at holding urine

ssure recordings of holding urine profiles re made in the control group and in the stress ontinent group pre and postoperatively.

At contraction the levator muscles compress the thra and elevate the bladder neck via the fascia vis. In the control group there were 2 women out of 7 whose levator contractions did not cause any nge in the profile. Five out of 10 women in the ncontinent group acted in the same way. Along the patients with good levator function the nges in the functional length and the maximal thral pressure were equal in both groups. Evn



Fig 7 The typical appearance of the urethral pressure profile at the different conditions shown in the figure

dently a good levator function can be compatible with severe stress incontinence. None of the stress incontinent women had experienced any improvement after half a year of exercise in holding urine. Thus it seems useless to try to improve severe stress incontinence in this way. But as it is important to have well trained levator muscles at the operation in order to get a strong muscle sling afterwards exercise in holding urine should be recommended for at least a short period preoperatively. After the operation 5 out of 10 women could contract their muscle slings as seen by the ability to influence the profiles. The urethral maximal pressure was increased as much as at holding urine preoperatively.

The functional length of the urethra was not increased to the same extent as before the operation. As the levators had been divided and rearranged they could not exert the same effect on the functional length through the elevation of the bladder neck.

Other changes induced by the operation

When dealing with incontinent patients one frequently finds urgency mingled with the stress component. Hitherto this has been regarded pretty much as a contraindication for surgical treatment. At this investigation a remarkable finding was that the operation seemed to increase the bladder capacity and make the patients feel light and severe tenesmus at higher bladder volumes than before (225–300 ml and 400–475 ml respectively). The reason could be the denervation at the operation and also the fact that the patients did not need a risk urinary leakage when the bladder was full postoperatively. Therefore they voided more seldom after the operation and their bladder capacity was normalized. Consequently even patients with a minor degree of urgency in their stress incontinence might be totally cured after pubococcygeal repair.

A possible risk with several types of operation for

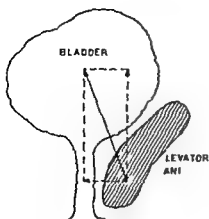


Fig. 8 The action of the levator ani on the urethra and the bladder

stress incontinence is to elevate the bladder neck too much with urinary retention as a result. Only one patient had a slightly increased residual volume (90 ml) 3 months after the pubococcygeal repair.

Theoretical and practical aspects of surgical treatment

Apparently the urethral pressure profile may give valuable information for the choice of operation method. If the urethral pressure before the operation is lower than normal it may be hazardous to use pubococcygeal repair or the Zoedler operation because the denervation of the bladder neck and the urethra may cause a further reduction. In cases a modified urethrocystopexia operation may be the method of choice. The dissection between the symphysis and the urethra should then be avoided as much as possible and only the bladder neck fixed to the symphysis.

This investigation has shown that the urethral pressure at rest is partly dependent on intact levator muscles. Division of these muscles eliminates part of the urethral pressure. Possibly this side effect is more than compensated by the supporting and compressing effect of the muscle sling on the proximal urethra at straining.

The risk for elongation and prolapse of the cervix is considerable if the bladder ligaments are sutured to the cervix whereby the force on the uterine suspension at straining becomes too great.

Suture of the undivided bulbocavernosus muscles did not contribute to the results and should be omitted. Perhaps a more lasting support under the distal urethra could be obtained if these muscles are di-

vided and arranged as a muscle sling under the distal urethra.

The epidural anaesthesia proved to be most suitable for this type of operation. Continence could be controlled by asking the patient to cough during the operation. Besides, the patients were spared from coughing and straining at the end of a general anaesthesia which could of course be a result by rupturing the sutures in the ligaments and the muscle sling.

CONCLUSION

This paper aimed at studying the changes in urethral functional length and pressure before and after pubococcygeal repair and these parameters pre and postoperatively in a continent control group. It has been shown that urethral functional length was markedly shorter, the pressure in the proximal part of the urethra lower in the incontinent women compared with continent ones. A remarkable finding was that the maximal urethral pressure did not differ between the two groups. The urethral pressure profile mostly had a quite different appearance with a sharply pointed pressure profile in the incontinent women compared with the continent women, whom a pressure plateau was the rule. 3 months after the pubococcygeal repair the pressure profile had assumed the pressure plateau appearance seen in the continent control group and on a markedly lower level. The functional length of the urethra was the same before and after the operation, i.e. 10 mm shorter than in the control group. At the operation the functional length was doubled and the maximal urethral pressure was increased. Both these changes were probably caused mechanically by dissection and compression, increase in the functional length and the maximal pressure did not persist after the operation, probably because of denervation, scarring and depression as the bladder ligaments slackened slightly.

Half of the operated patients were able to retract their pubococcygeal muscle slings and to prominently increase the urethral pressure to the same extent as in the continent women.

The restoration of the pressure plateau was only change in the pressure profile which persisted after surgical treatment. Perhaps it is secondary

malized position of the bladder neck and an un-
 ved tone of the proximal urethra which possibly
 of importance for the maintenance of continence
 o circumstances have been shown to be of com-
 ed importance for the transformation to conti-
 nence and the continent condition namely the ele-
 ved position of the bladder neck and the good
 re in the most proximal part of the urethra seen
 the urethral pressure profile. To get further from
 standpoint in understanding the genesis of un-
 y stress incontinence it seems essential to
 uate the changes in the pressure of the urethra
 the bladder also in a dynamic situation.

REFERENCES

- Asmussen M & Ulmsten U. A new technique for
 measurements of the urethral pressure profile. *Acta*
Obstet Gynecol Scand 54: 385 1975
- Asmussen M & Ulmsten U. Simultaneous urethro-
 cystometry with a new technique. *Scand J Urol*
Nephrol 10: 7 1976
- Johnson G. Simultaneous recording of intravesical
 and intra urethral pressure. *Acta Chir Scand Suppl*
 476: 1 1961

- 4 Hodgkinson C P, Drukker H H & Hershey G J
 C. Stress urinary incontinence in the female. VIII
 Etiology. Significance of the short urethra. *Am J Ob-*
stet Gynecol 86: 16 1963
- 5 ICS Standard Committee 1975. The fifth annual meet-
 ing of ICS. Glasgow 1975
- 6 Ingelman Sundberg A. Urin inkontinens hos kvin-
 nan. *Nord Med* 50: 1149 1953
- 7 Lapides J, Ajemian E F, Stewart B H, Breakey
 B A & Lichtwardt J R. Further observations on the
 kinetics of the urethro-vesical sphincter. *J Urol* 84: 86
 1960
- 8 Lund C J, Fullerton W E & Tristan T A. Cine-
 fluorographic studies of the bladder and urethra in
 women. *Am J Obstet Gynecol* 78: 706 1959
- 9 Ulmsten U, Asmussen M & Lindstrom K. A new
 technique for simultaneous urethrocystometry includ-
 ing measurement of the urethral pressure profile. The
 fifth annual meeting of ICS. Glasgow 1975

Submitted for publication Feb 27 1977

A Öbrink
 Department of Obstetrics and Gynecology
 Sabbatsberg Hospital
 Stockholm
 Sweden

SOME METHODOLOGICAL ASPECTS ON THE MEASUREMENT OF INTRALUMINAL PRESSURES IN THE FEMALE UROGENITAL TRACT IN VIVO

Kjell Landstrom and Ulf Ulmsten

From the Department of Biomedical Engineering and the Department of Obstetrics and Gynecology University Hospital of Malmö Malmö Sweden

Abstract This short survey presents some methodological aspects on intraluminal pressure recordings *in vivo* from the female urogenital tract. The importance of adequate calibration is stressed and a calibration unit for different types of recording equipments is described. Calculations measuring errors are outlined. The selection of recording catheters is discussed from both the theoretical and the practical point of view. Finally some practical aspects are considered on pressure recordings *in vivo* from the ureter, bladder, the urethra and the uterus. This discussion is illustrated by pressure diagrams obtained with different recording techniques from the actual organs.

Measurement of intraluminal pressure has been widely used for assessing the function of the urogenital tract. However, owing to the inaccuracy of the methods used, the results have often been inconsistent and difficult to interpret. Rigorous demands are placed on the measuring equipment, especially relating to the properties of the catheters. Stiff catheters can cause damage or artificial constriction of the walls of the organ investigated, resulting in erroneous recordings as a result. On the other hand, the necessity of using relatively long catheters for pressure recordings in the urogenital tract implies a risk of defective pressure transmission in the measuring system, especially when conventional open-end fluid-filled catheter systems are used.

In order to avoid or at least minimize technical measuring artefacts, the intended equipment must be thoroughly checked *in vitro* in order to find out whether it has sufficient capacity to record the pressure variations in the organs in question. This requires a reliable calibration instrument implying the possibility of calibrating the measuring equipment *in vitro* and also the possibility of calculating

the magnitude of the potential technical errors during *in vivo* measurements. Such a calibration unit is described in Fig. 1. It offers the possibility of testing practically all types of presently available catheter recording systems.

As a general rule of thumb, the rise time (the inertia) of the measuring system should be three times faster than that with which the physiological rises in pressure occur. If the physiological

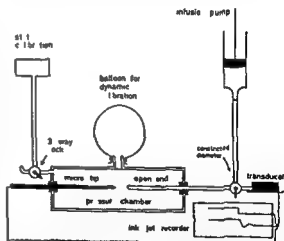


Fig. 1 Calibration unit used for static and dynamic calibration of different pressure recording systems. The pressure chamber is situated in the middle of the figure. At the upper part of the chamber there is a balloon which can be inflated by air, thus causing an optional pressure within the pressure chamber. In this figure two pressure recording systems are tested: (Left) a micro-tip-transducer; (right) an open end catheter connected to a conventional transducer. Static calibration can be performed, since the pressure chamber is connected to a water column (upper left).

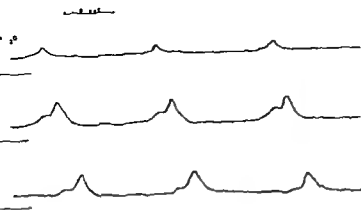


Fig 3 Normal antegrade ureteral peristalsis recorded with a central-hole catheter as described in Fig 2. The upper trace reflects the pressure 25 cm from the bladder, the middle trace the pressure at 11 cm and the lower trace the pressure 5 cm from the bladder. The first rise in pressure as seen most markedly in the middle and the lowermost tracings indicates a bolus of urine. The contractions start at upper part of the ureter.

What static and dynamic properties should the measuring system possess in order to allow reliable measurements in the organs examined? Does our recording system meet these requirements? Expressed in other words: what measuring errors must be expected and within what limits are the measurements valid?

The above questions can be answered with the help of a reliable calibration unit. But there is also a third question to be answered, which is most important: To what extent is the patient exposed to risks by the intended measurements? This question must be carefully assessed from an ethical point of view and before any measurements are made the investigator must inform the patient about the nature of the examination and obtain her consent.

Technical applications

We will close this brief description by demonstrating some pressure diagrams obtained with our different

techniques for pressure recordings in the female urogenital tract.

When recording the intraluminal pressure in the ureter it is of great importance that a thin catheter does not obstruct the flow of urine through the organ. However, the intra-ureteral recording is of minor value if the pressure measurements are not performed on at least three intra-ureteral levels. Otherwise it is not possible to calculate the direction and rate of propagation of the peristaltic waves. The solution of this pressure recording problem is to perform recordings with a central hole catheter (Fig 2). Fig 3 shows an intra-ureteral pressure recording with such a catheter in a patient with normal antegrade ureteral peristalsis. In Fig 4 retrograde ureteral peristalsis is demonstrated in a pregnant woman admitted to the hospital because of pain in the right flank.

Pressure recordings from the lower urinary tract can be very helpful in diagnosing different types of urinary incontinence. To obtain a reproducible

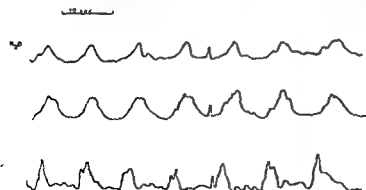


Fig 4 Retrograde peristalsis in a ureter from a pregnant patient. The same recording catheter and recording levels as described in Fig 3. The ureteral contractions start at the lowermost recording level, i.e. nearest the bladder. At the arrow the patient coughs, which indicates that the pressure is transmitted equally from the three recording channels.

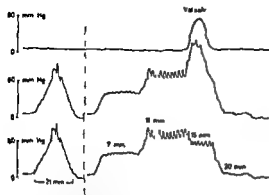


Fig 5 Continuous urethral pressure profile obtained with a micro-transducer catheter. The top trace indicates the bladder pressure, the middle trace the maximal urethral pressure and the lowermost trace the difference between the urethral pressure and bladder pressure, i.e. the urethral closure pressure. At the beginning both the transducers (Fig 6) are situated within the bladder. The catheter is then withdrawn from the urethra at a constant rate. By this manoeuvre the intra urethral pressure is recorded throughout the entire length of the urethra, giving the urethral pressure profile as seen in middle trace, left part of the diagram. In the right part of the figure the procedure is repeated but the withdrawal is halted at different sites in the urethra. It is then seen that when the catheter is halted in the middle part of the urethra at about 11 mm from the inner meatus the highest intraluminal pressure is recorded and at this site marked pulsations synchronous with the patient heart beats are present. Finally it can be seen that a Valsalva manoeuvre affects the bladder and urethral pressure equally.

urethral pressure profile (Fig 5) and to be able to record the rapid pressure variations in the bladder and the urethra caused by coughing or other intra abdominal pressure increases (which may occupy less than 50 msec) it is necessary to use a pressure recording system with a high frequency response. The microtransducer catheter (Fig 6) offers the best solution for this recording problem. It must be emphasized that pressure recording in the lower urinary tract should offer the possibility to record the bladder pressure and the maximal urethral pressure simultaneously—otherwise it is difficult to evaluate functional disorders in the lower tract. Fig 7 demonstrates normal micturition in a young healthy female. As seen there is at the initiation of micturition a rapid fall in the intra urethral pressure and later on there is an increase in intravesical pressure indicating activation of the detrusor. By electronic subtraction of the bladder pressure from the maximal intra urethral pressure it is possible to obtain the urethral closure pressure. A positive closure pressure indicates continence, whereas a closure pressure of zero or below indicates incontinence, as seen in Fig 8.

The uterine muscle contracts slowly and for relatively prolonged periods. This means that no demands on the frequency response of the recording system is necessary. However, as re-

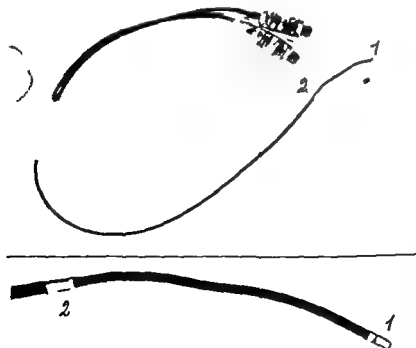


Fig 6 Micro-transducer catheter. The pressures are recorded with two micro-transducers. These are enclosed in a Dacron sheath 1 cm apart. The area of the catheter is only 0.75 mm². At the bottom of the figure the catheter is shown about four times enlarged.

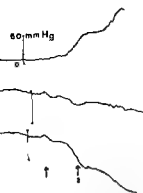


Fig 7 Initiation of micturition in a healthy female (micro-transducer catheter) Bp=bladder pressure Up=urethral pressure Cp=urethral closure pressure At arrow 1 the patient is requested to micturate and there is a marked fall in the urethral pressure and the closure pressure. At the arrow 2 the closure pressure reaches zero and urine begins to escape from the urethra

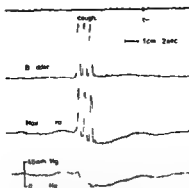


Fig 8 Pressure recordings from a patient suffering from stress incontinence. Micro-transducer catheter same recording technique as illustrated in Fig 7. When the patient coughs the closure pressure falls to zero and a small amount of urine escapes from the urethra

ed recordings are necessary to characterize myometrial activity this aspect places high demands on the function of the recording equipment. We found that the micro-transducer technique as used at present seems to be the best method for prolonged intra uterine recording in the non-pregnant uterus (10).

So far most pressure recordings from the non-pregnant uterus have been performed during static conditions but a recently introduced technique allows to offer possibilities to perform dynamic studies of the intact human myometrium (5).

In the pregnant uterus great interest has been focused on myometrial activity during labor and extensive work has been done on this subject in this country (3, 4, 6, 7). It is not possible to quantify myometrial activity by means of external tocography. Intra uterine pressure recordings via cervically introduced recording catheters involve the risk of rupturing the membranes and therefore cannot be used if it is not intended to deliver the patient in conjunction with making the recordings. We have to admit that recording intra uterine pressure of the pregnant uterus is difficult as yet no ideal method exists (8). There is a considerable need for more accurate non-invasive methods. In the development of such methods the most accurate present available technique for correlating myometrial activity during labour must be used as reference. In our opinion this implies the use of simultaneous ultrasound measurement and

intra uterine pressure recordings with the micro-transducer technique (8). From such an arrangement the tension of the uterine muscle can be calculated with a relatively high degree of accuracy.

Combined pressure recording and ultrasound measurement can also be used to calculate the effect of uterine contractions on the cervix (8).

REFERENCES

1. Asmussen H, Lindström K & Ulmsten U. A catheter manometer calibrator. A new clinical instrument. *Biomed Eng* 10: 175, 1975.
2. Bengtsson L, Ph. The sponge tipped catheter. A modification of the open end catheter for recording of myometrial activity in vivo. *J Reprod Fertil* 16: 115, 1968.
3. Ingelman Sundberg A, Lindgren L & Ljungström T. An electronic method for intra uterine measurement of pressure during labor. *J Obstet Gynaecol Br Comm* 60: 372, 1953.
4. Ingemarsson I. Inhibition of myometrial activity during human pregnancy by beta adrenoceptor stimulation. *Studentlitteratur* Lund 1975.
5. Joelsson J, Gidlund L, Anzén B & Ingelman Sundberg A. In vivo determination of the stress-strain relation of the human myometrium. *Acta Obstet Gynecol Scand* 55: 325, 1976.
6. Lindgren L. Ist die normale Uterusmotilität Voraussetzung zur Erzielung einer schnellen Cervixdilatation. *Arch Gynaekol* 197: 494, 1977.
7. Landmark G. Induction of labor with prostaglandine $F_{2\alpha}$ by intravenous infusion. *Acta Univ Uppsals* 228: 1975.

- 8 Lindstrom A. & Ulmsten U. Registrering av livmoderkontraktion, extern tochografi och intrauterin tryckmatning. Övervakning under förlossning. Spr. Seminarium November 1976.
- 9 Ulmsten U. Studies on ureteral function in vivo. Studentlitteratur Lund, Sweden 1974.
- 10 Åkerlund M., Bengtsson L. Ph. & Ulmsten U. Recording of myometrial activity in the nonpregnant human uterus by a micro-transducer-catheter. *Biophys. Reprod.* 1976 (in press).

Submitted for publication Feb. 27 1977

U. Ulmsten
Dept. of Obstetrics & Gynaecology
Allmänna Sjukhuset
S-214 01 Malmö
Sweden

FOLLOW UP STUDIES IN DYSPLASIA AND CANCER IN SITU OF THE CERVIX UTERI

Vlasta Vaclavinkova Anna Kristina Hedman and Karen Naselli

*From the Department of Obstetrics and Gynecology Karolinska Institutet
Stockholm Sweden*

Abstract In order to study when recurrences appear following conization for dysplasia or cancer in situ of the cervix uteri a series of 477 patients has been investigated. All underwent conization at Sabbatsberg 1965-1970. Among the 181 cases of dysplasia two developed recurrences one respectively three years after treatment. In the series of 296 patients with cancer in situ thirteen recurrences appeared. Eight of them were detected within the three years. The other five were spread out during the four years. About 50% of all patients had been followed up for five years or more (Table I). The conclusion is reached that a yearly follow-up is necessary for at least ten years. As it is impossible to take care of such an increasing number of examinations at the Swedish departments of obstetrics and gynecology without extra personnel the following proposal is made: During the first five years after treatment the examinations are performed at the department where the conization has been performed. After that period the follow up is limited to vaginal smears taken once every year in connection with the general gynecologic health control.

Following preliminary studies (3-4) systematic gynecologic screening started in Sweden 1966. Vaginal smears were taken by midwives to begin with in women in age groups at maximal risk and then successively including both older and younger women. The aim was that a smear should be taken in every woman every fourth year. In spite of the fact that only one third of the counties took part in the campaign in the beginning the number of recorded cases of cancer in situ was doubled during the first two years. The result has been a steadily increasing number of patients in whom regular follow up examinations are needed after conization, imposing a heavy burden for the different departments of obstetrics and gynecology. The routine at present seems to be that the patients are examined at least once every year. A study has therefore been

made in order to investigate if the interval between the follow up examinations could be extended after a certain number of years.

MATERIAL

181 cases of dysplasia of the cervix and 296 patients with cancer in situ treated by conization between 1965 and 1970 at the Sabbatsberg department of obstetrics and gynecology have been studied. They have regularly been followed-up with colposcopy and smears at a special weekly clinic by a doctor trained in colposcopy. Unfortunately however several of them have been lost during the time of observation. Thus all have been followed up for at least one year but only about 50% for five years or more. The age distribution in the group is shown in Fig. 1.

METHODS

When the diagnosis of dysplasia or cancer in situ had been obtained on biopsies usually directed with the help of the colposcope a conization was made. The aim was to make a longer cone in postmenopausal women than in those of fertile age and to perform a curettage of the rest of the cervical canal. As however the operation was performed by several different young colleagues the technique and accuracy may have varied.

The pathologic examination was made at the Sabbatsberg Institute of Pathology on eight evenly distributed tissue slices cut radially around the cervical canal through the whole length of the cone. Histologically radical extirpation of the lesion was obtained in 90.1% of the cases of dysplasia and in 81.1% of those with cancer in situ. If the smear was completely negative one month after the operation no repeat conization was performed even if the cone had been incomplete. Otherwise repeat conization was undertaken.

Those cases were classified as recurrences where atypia appeared histologically after at least one year of negative colposcopy and smears.

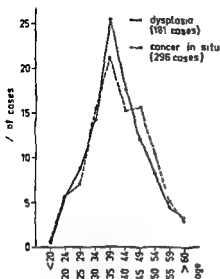


Fig 1 Age distribution

RESULTS

Among the 181 cases of dysplasia recurrences appeared only in two cases (Table I) one and three years after the conization respectively. In both cases dysplasia was present in the margin of resection. The recurrence was classified as cancer in situ in both cases.

Among the 296 cases of cancer in situ thirteen patients had recurrences two as late as six and seven years after the conization (Table I). In three of them the cones had been judged histologically as complete and in ten cases cellular atypia was present in the margin of resection. All had their primary cancer in situ at the squamo-columnar junction. In nine patients the recurrences were classified as dysplasia and in four as carcinoma in situ. No case of invasive carcinoma was diagnosed.

In two patients carcinoma of the endometrium was diagnosed four and eight years respectively after the conization and one was operated on for an ovarian carcinoma ten years after the primary operation.

DISCUSSION

An adequately performed radical conization is an excellent treatment for dysplasia as well as for cancer in situ of the cervix but is no guarantee of cure as three of our recurrences appeared in such cases. However several authors state (1, 2, 5, 6)

that they have never observed a recurrence of radical conization whereas Zippel (7) had observed recurrences as late as fourteen years after conization. The latter observation seems to be local as a conization can never remove local predisposing factors for the development of cancer. The appearance of other types of genital cancer in three women of this series during the time of observation may also indicate a general disposition to cancer among women with dysplasia or cancer in situ of the cervix. A careful follow up is therefore necessary.

CONCLUSIONS

In our series the recurrences are spread out over more than seven years as seen from Table I. Most of them however appear within the first three years after the conization.

As it is important to diagnose a recurrence early as possible follow up examinations every year seem to be necessary for a period of at least ten years.

The accumulation of new cases every year makes it however impossible for the present staff at Swedish departments of obstetrics and gynecology to carry out all these examinations. It is therefore proposed that the follow up during the first three years takes place in the department where the conization has been made. After that period the examinations should be limited to vaginal smears to be done once every year by midwives who operate no gynecologic screening tests.

Table I Recurrence frequency during 11 years observation

Follow up after years	Dysplasia		Cancer in situ	
	No of cases	Recurrences %	No of cases	Recurrences
1	181	1	196	2
2	169	0	184	5
3	151	1	250	1
4	136	0	211	1
5	96	0	170	2
6	82	0	171	1
7	40	0	86	1
8	28	0	64	0
9	19	0	30	0
10	14	0	12	0
11	1	0	2	0

REFERENCES

- Burghardt E. Bemerkungen zu der Publikation von F. Dachmann über Das Rezidiv des Ca in situ der Zervix. *Geburtsh Frauenheilk* 33: 367 1973
- Held E. Prophylaxe und Früherfassung des Kol-
lomecarzinoms. *Praxis* 28: 1071 1970
- Ingelman Sundberg A. Gynekologisk hälsokontroll.
Riksföreningen mot cancer. Årsbok 1960-1967
p 461-464
- Gynecological Health Control. INSERM
p 71-74 Paris 1972
- Krimmenau R. Fehler die zum Rezidiv nach fest-
gestelltem gesteigert dysplastischem Epithel (Carcinoma in
situ) führten (1950-1965). *Zschr Arztl Fortbild* 3: 170
1973
- 6 Neubert C. Restbefunde nach diagnostischen Konisa-
tion. *Geburtsh Frauenheilk* 28: 478 1968
- 7 Zippel H. H. Citoler P. Zippel C. Rezidiv Quote
bei nicht im Gesunden entfernten Carcinoma in situ der
Zervix. *Geburtsh Frauenheilk* 34: 369 1974

Submitted for publication Nov. 18 1976

Vlasta Václavíková
Department of Obstetrics and Gynecology
Sabbatsberg Hospital
11387 Stockholm
Sweden

PREMALIGNANT AND MALIGNANT UTERINE CHANGES IN IMMUNOSUPPRESSED RENAL TRANSPLANT RECIPIENTS

Hugo Husslein Gerhard Breitenacker and Gerhart Tatra

*From the 2nd Department of Obstetrics and Gynecology University of Vienna Medical School
Vienna Austria*

Abstract 29 female immunosuppressed renal transplant recipients were examined gynecologically. In 2 cases the helium of the portio was found to be dysplastic while metrical carcinoma was present in 1 patient. A review of literature suggests that immunosuppressed patients are more likely to develop tumors than others. The authors stress the need for gynecological and cytological examinations at short intervals to identify premalignant early malignant uterine changes at a time at which they can readily be treated without discontinuation of the immunosuppressive therapy.

Although the techniques for tissue typing have become increasingly sophisticated, recipients of organ transplants still have to undergo prolonged and specific immunosuppressive therapy to reduce the effects of host transplant incompatibilities and as a result increase the chances for successful organ transplantation. Immunosuppression is currently achieved with ionizing radiation, cytostatics (alkylating agents, antimetabolites, antibiotics, etc.), corticosteroids and antilymphocyte sera. However, the resultant interference with the organism's immune responses is associated with undesirable side effects including primarily an increased susceptibility to infections. Observations made in the past few years appear to suggest an accumulated incidence of malignancies (de novo tumor growth) in immunosuppressed renal transplant recipients who in turn account for the major share of all transplant recipients. The genital region has been found to be one of the areas affected. This prompted us to develop our own material of female immunosuppressed renal transplant recipients gynecologically.

MATERIAL AND METHOD

In the past 12 months 29 female renal transplant recipients underwent gynecological examinations. The patients' age ranged from 13 to 50 years (mean age 33 years). Kidney transplantation had been performed 2 to 100 months (mean 22.5 months) ago. All patients have received post-transplantation immunosuppressive therapy. Current medication consists of antimetabolites in 26 cases, alkylating agents in 4 cases, and corticosteroids in 27 cases.

Follow-up studies included gynecological examination, colposcopy, microbiological investigation of Gram-stained smears and cytology of cervical smears. Histological studies were done in 3 cases. In 2 with positive cytology on cone biopsies processed by serial step sections and in 1 on curetted material and after extirpation on the entire uterus.

RESULTS

(a) Clinical gynecology and colposcopy

On palpation 22 females were normal while the uterus in the remaining cases was found to be appreciably smaller than normal. The lower pole of the transplant kidney was consistently palpated in a left or right parauterine location. There was no evidence of adnexal pathology or parametrial infiltration. In 1 case extensive recurrent vulvar, vaginal and perianal condylomata acuminata was present.

Colposcopy of the portio brought to light a great many abnormalities. Coalescent and disseminated iodine-negative areas of variable extension in an otherwise normal epithelium constituted the most common finding, such areas being present in 14 cases. Ectopia was found in 8 females, a transformation zone in 5. Four patients showed true erosion while 6 had additional slight leukoplakia. Normal portio epithelium was only seen in 4 cases.

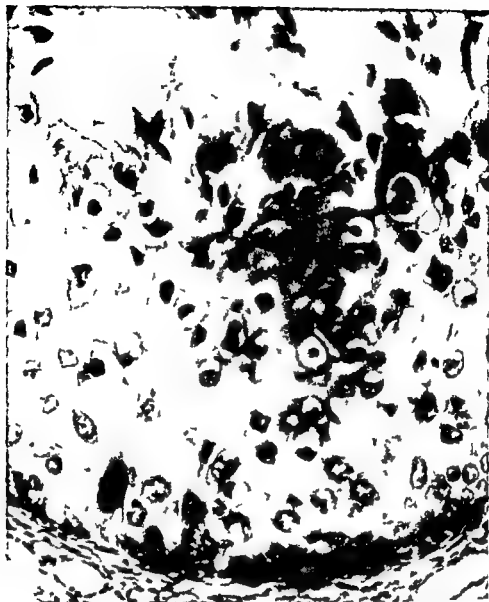


Fig 1 Dysplastic squamous epithelium at the uterine cervix 24 months after kidney transplantation H & E $\times 100$

(b) Microbiology

In 2 cases gram stained smears were clear of microorganisms on microscopy. Mixed bacteria were present in 20 cases, fungi in 15. *Trichomonas* associated with *candida albicans* were found in one female.

(c) Cytology and histology—case reports

Cervical smears were negative cytologically in 27 cases. Three females showed premalignant or malignant uterine changes.

Case 1

This 32 year-old female had received a kidney 24 months previously. The smear showed abundant karyotic squamous cells. Consistent with histological evaluation. The distal cervical canal showed areas of atypically proliferating squamous epithelium with abundant cellular atypia, highly polymorphous nuclei, and abnormal mitoses extending into the epithelial layers. The stratification of the epithelium still maintained, the epithelial cells were rich in plasma. This dysplastic epithelium was also found in the glands. The epithelio-stromal junction was well served (Fig 1). Excision of this atypical epithelium extended well into intact tissue. Still a typical cell



2 Normal endometrium in material obtained by curettage from a 44 year-old patient 8 months after kidney transplantation. H & E $\times 59$

was not visible on cytology 6 weeks after conization. Colposcopy of the portio showed leukoplakia with an iodine negative area. The patient was followed up cytologically and colposcopically at short intervals.

Case 2

A 30-year-old female received a transplant kidney at another hospital 8 years ago. Thirty-six months prior to transplantation, somewhat atypical cells were first found on cytology. From then on smears were taken at intervals of 6 months. Since atypical cells were found 10 months after conization was done. On histology dysplastic epithelial areas were seen around the last cervical gland as well as in the squamous epithelium on the ectocervix extending down to the vaginal resection line. Cytologic follow-up studies again revealed atypical cells. On colposcopy extensive iodine negative areas extending into the portio and a slight leukoplakia of the portio were seen. Follow-ups are continued at short intervals.

Case 3

A 44-year-old female underwent renal transplantation 8 months before curetting was performed for metrorrhagia.

The histological examination of the curetted material revealed endometrial tissue with loss of function. There was however no evidence of malignancy (Fig. 2). Twelve months later, i.e. 20 months after transplantation, a polypoid structure protruding from the cervical canal was removed and the curettage repeated. The endometrial tissue obtained showed areas of highly differentiated adenocarcinoma. These prompted total extirpation of the uterus. Both the endometrium and the mucosa along the isthmus of the uterus were largely replaced by highly differentiated adenocarcinomatous tissue infiltrating the innermost myometrial layers (Fig. 3).

DISCUSSION

Ionizing radiation, antiproliferative substances (cytostatics), corticosteroids and antilymphocyte sera can be used to suppress immune responses to transplants. Generally the suppressive agents available are used in combinations. However the resultant interference with the organism's immune

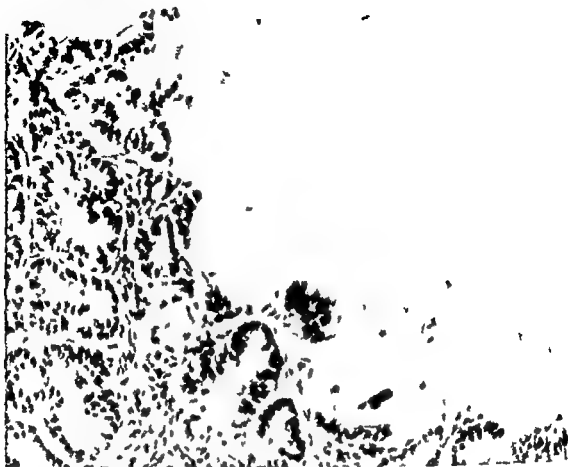


Fig 3 Same patient as in Fig 2 12 months later highly differentiated endometrial adenocarcinoma H & E $\times 59$

mechanisms causes a number of undesirable side effects (1) the most common and most dreaded being a reduced resistance to bacterial (viral early herpetic) and mycotic infections. 30% of posttransplantation deaths are due to complications (2). In our 29 cases more or less pronounced signs of an inflammatory process were present in 27 smears. Mycotic infections predominating with an incidence of 15. This compares with an incidence of only 1% in the total population of our department. *Trichomonas* normally among the most common organism encountered in the vagina was present in only 1 case. In another case vulvar vaginal and perianal condylomata acuminata were seen. This is apparently suggestive of a viral infection.

The mutagenic and oncogenic potential of cytostatic substances such as used for immunosuppressive therapy is well documented. Alkylating agents are for instance known to have a

carcinogenic effect. This is explainable by interfering with the synthesis and/or structure of nucleic acids. A potential carcinogenicity of antibiotics and antimetabolites is discussed (3).

Aside from the direct cellular effect is the immunosuppressive agents the reduced immunological response to spontaneous mutations and oncogenic viruses as well as the continuous genic stimulation of the RES by allografts vs host reaction) may be factors underlying the accumulated incidence of tumors in immunosuppressed patients (6). All of this may potentiate the effects of known carcinogenic agents (7). Taken individually a single therapy modality is thus not very likely to increase cancer hazard. But immunosuppressive therapy collectively been incriminated as a factor underlying the reduced response of the organism to cancer i.e. the immuno surveillance (8). Pretransplantation suppression of immunologic responses

reumatic condition of renal transplant recipient may be a compounding factor (9). Immunosuppressed patients the risk of developing cancer is 50 to 100 times higher than in an age-matched group of comparable age. Enderlin & Starzl (6) found 3 tumors (4.6%) in 65 transplant recipients of the 352 renal transplant cases studied by Penn & Starzl (7). 17 had tumors. When excluding those patients who had died by the 4th postoperative month, the figures are 5 and 5.6% respectively. Correcting their figure for non-survivors Wegmann et al (9) found 16 de novo tumors in 190 cases. The incidence reported by Birkeland & Kemp (10) in a material of 100 cases was however only 0.75%. In a control group of comparable age, by contrast, the incidence was no more than 0.058% (7).

Of the de novo tumors, 62% are epithelial and mesenchymal. Sarcomas of the lymphoreticular system (29%) are significantly common, about 10% of them localizing in the CNS and the spinal cord. Skin and lip tumors equally account for

the average age of patients with epithelial tumors (50 years) is higher than for mesenchymal tumors (40 years). Mesenchymal tumors are found to occur earlier after transplantation (22 months) than epithelial tumors (33 months) following the transplantation (on an average). Unlike mesenchymal tumors, carcinomas localizing at the body surface have a better prognosis (11).

The first cervical carcinoma following a kidney transplantation was reported by Tallent et al in 1961 (12). Since that time the Denver transplant center alone had registered 18 cervical carcinomas by March 1974. These account for 8% of the de novo tumors recorded. Of the Denver cases, 16 were preinvasive and 2 were invasive. The patients' age was 22 to 50 years (mean 36 years), the posttransplantation interval being 6 to 97 months (mean 38 months). Pretransplantation cytology had only been done in 5 cases, all of them being negative. Statistical computation showed that immunosuppressed patients are 14 times more likely to develop carcinoma of the cervix (13) than

in our material of 29 female renal transplant recipients. 3 had squamous dysplasia of the cervix. Although in one case the excision of the dysplastic epithelium had extended far into intact tissue, subsequent smears failed to be negative. Post-

transplantation colposcopies again revealed negative areas of variable dimensions and focal leukoplakia. On cytology, atypical cells were again seen. The disseminated occurrence of areas of atypical epithelium might suggest that in association with reduced immune response these changes develop not only at the squamo-columnar junction but also at multiple sites of cervix and vagina.

While carcinoma of the cervix has repeatedly been reported to develop in immunosuppressed patients, the literature available to us lists only 1 case of posttransplantation endometrial carcinoma (14). The case presented by us appears to be of particular interest, as the curettage performed 1 year prior to the diagnosis of endometrial carcinoma had been negative.

Both the reports in the literature and our own observations document the need for gynecological and cytological follow-up studies at short intervals in all females undergoing immunosuppressive therapy after organ transplantation. These follow-up studies at an interval of 6 months usually help to detect premalignant or early malignant growths in the genital tract at a time at which these can readily be treated without discontinuing immunosuppressive therapy.

REFERENCES

- 1 Hartwich G. Nebenwirkungen zytologischer und immunsuppressiver Therapie. *Fortschr Med* 91: 357, 1973.
- 2 ACS/NIH Organ Transplant Registry. The Tenth Report of the Human Renal Transplant Registry. *J Am Med Ass* 221: 1486, 1972.
- 3 Schmahl D. Karzinogene Wirkung von Cyclophosphamid und Thiazichon bei Ratten. *Dtsch Med Wschr* 92: 1150, 1967.
- 4 Schmahl D. Experimentelle Untersuchung über karzinogene Wirkungen von Krebs-Chemo-Therapeutica und Immunsuppressiva. *Arzneim Forsch (Drug Res)* 20: 1461, 1970.
- 5 Hartwich G. Zytostatika Therapie. Erwünschte und unerwünschte Wirkungen. *Fortschr Med* 91: 1218, 1973.
- 6 Enderlin F & Guisan Y. Maligne Tumoren unter Immunsuppression: ein neues Problem beim Transplantierten. *Schweiz Rundschau Med* 62: 1031, 1973.
- 7 Penn I & Starzl Th E. Immunsuppression and Neoplasia. *Behring Inst Mitt* 51: 204, 1977.
- 8 Burnet F M. Zit in Micksche M. Beeinflussung des Immunstatus von Krebspatienten durch Chemotherapie. *Wien Med Wschr* 125: 779, 1975.
- 9 Wegmann W, Lärpader F & Bräswanger U. Maligne Geschwülste nach Nierentransplantation. *Schweiz Med Wschr* 104: 809, 1974.

- 10 Birkeland S A & Kemp E Malignant tumours following immunosuppression in renal transplantation EDTA Proceedings pp 429-433 1973
- 11 Penn I & Starzl Th A summary of the status of de novo Cancer in transplant recipients Transplant Proc 4 719 1972
- 12 Tallent M B Simmons R L & Najarian J S Primary carcinoma of the cervix appearing in immunosuppressed renal transplant recipient Am J Obstet Gynecol 109 663 1971
- 13 Porreco R Penn J Droegemueller W Greer B & Makowski E Gynecologic malignancies in immunosuppressed organ homograft recipients Obstet Gynecol 45 359 1975
- 14 Kim H & Williams R J Endometrial carcinoma of the uterus and ovaries associated with immunosuppressive therapy and anticoagulation: report of a case Mayo Clin Proc 47 39 1972

Submitted for publication Nov 3 1976

H Husslein
II Universitäts Frauenklinik
Spitalgasse 23
1090 Vienna
Austria

REGRESSION OF TUMOUR GROWTH AFTER ADMINISTRATION OF ALKOXYGLYCEROLS

Astnd Brohult Johan Brohult and Sven Brohult

m Radiumhemmet Karolinska sjukhuset Stockholm 60 Medical Department IV Södersjukhuset Stockholm 38
and the Royal Academy of Engineering Sciences Stockholm Sweden

act A regression of tumour growth is observed
alkoxyglycerols are administered prior to radiation
ment of patients suffering from cancer of the uterine
x This regression has been demonstrated by a
ge in the quotient between the incidence of early and
nced stages

xyglycerols occur in small quantities in many
ral products In the haemopoietic organs of
imals particularly the bone marrow they are
ively abundant They are also found in rela-
y high concentrations in human mother's milk
/ occur most abundantly in nature in the liver
f certain species of shark (1-8-9) The general
ula for alkoxyglycerols is $\text{CH}_2\text{OH}-\text{CHOH}-\text{R}$
O R where R is a long chain aliphatic radical
e alkoxyglycerols have proved to be of medi-
interest (1-7) To some extent they prevent
openia and thrombocytopenia The administra-
of alkoxyglycerols before during and after
tion treatment of patients with cancer of the
ne cervix results in higher survival rates than if
tion treatment alone is given (1-2) Further-
the alkoxyglycerols promote the growth of
obacillus lactis (1) the formation of antibodies
1 and they reduce to a large extent (ca 50%)
equency of injuries following radiation therapy
(10)
e aim with the present study has been to in-
gate the regression of tumour growth when
xyglycerols are administered before radiation
ment of patients suffering from cancer of the
ne cervix

MATERIALS AND METHODS

linical experiments in this study were conducted
alkoxyglycerol preparations from the liver oil of the

Greenland shark The preparation produced by AB Astra
with the working name AT III is a concentrate contain-
ing 85% free alkoxyglycerols The contents of various
alkoxyglycerols from a variety of sources are given in
Table I

The alkoxyglycerols were administered orally in
capsules 2 capsules 3 times a day each capsule contain-
ing 0.1 g of alkoxyglycerols The total daily dose thus was
0.6 g

The practical procedure was as follows Immediately
upon the receipt of the referral letter the patient was
given a date for the commencement of radiotherapy and III
the same time a package containing the alkoxyglycerols
and information regarding the dosage At the start of

Table I Percentage composition (weight) of al-
koxyglycerols from various sources

Alkoxy glycerols	Human bone marrow	Human milk	Liver oil Greenland shark
14:0			2.0
15:			0.7
16:0	79.4	23.9	9.1
16:1		trace	10.8
17:	7.6	3.6	3.6
18:0	4.6	22.8	2.8
18:1	16.7	33.8	59.4
18:2		1.4	1.6
18:3			
19:	6.1	2.4	1.5
20:0	2.9	1.6	
20:1	3.2	2.3	6.2
22:0	0.7	0.7	
22:1	5.1	3.4	2.2
24:		2.1	

Analyses are according to Hallgren & Larsson (7-3)
The number of carbon atoms in the first column refers to
the long-chain component of the molecule The number
after the colon denotes the number of double bonds
Both branched and normal chains C_{14} , C_{17} and C are
present

- 10 Birkeland S A & Kemp E Malignant tumours following immunosuppression in renal transplantation EDTA Proceedings pp 429-433 1973
- 11 Penn I & Starzl Th A summary of the status of de novo Cancer in transplant recipients Transplant Proc 4 719 1972
- 12 Tallent M B Simmons R L & Najarian J S Primary carcinoma of the cervix appearing in immunosuppressed renal transplant recipient Am J Obstet Gynecol 109 663 1971
- 13 Porreco R Penn I Droegemueller W Greer B & Makowski E Gynecologic malignancies in immunosuppressed organ homograft recipients Obstet Gynecol 45 359 1975
- 14 Kim H & Williams R J Endometrial carcinoma of the uterus and ovaries associated with immunosuppressive therapy and anticoagulation: report of a case Mayo Clin Proc 47 39 1972

Submitted for publication Nov 3 1976

H Husslein
II Universitäts Frauenklinik
Spitalgasse 23
1090 Vienna
Austria

Table IV Distribution according to clinical stages (1958-1975) Stage I A excluded

	I B		II A		II B		III		IV		I B-IV	
	n	%	n	%	n	%	n	%	n	%	n	%
Radiotherapy												
-64	646	25.1	806	31.3	564	21.9	384	14.9	176	6.8	2576	
-75	393	27.1	408	28.1	329	22.7	225	15.5	95	6.6	1450	
d	1039	25.8	1214	30.2	893	22.2	609	15.1	271	6.7	4026	
Prophylactic administration of alkoxyglycerols												
ad 1	159	38.5	129	31.2	71	17.2	39	9.5	15	3.6	413	
ad 2	36	30.0	39	32.5	19	15.8	18	15.0	8	6.7	120	
ad 3	84	36.7	78	34.1	34	14.8	26	11.3	7	3.1	229	
d	279	36.6	246	32.3	124	16.3	83	10.9	30	3.9	762	

Table V A Decrease in advanced stages after prophylactic administration of alkoxyglycerols (all stages)

p	No of patients	E		A		E/A	D (%)	D _A (%)
		n	%	n	%			
Radiotherapy (8-75)	4400	2627	59.7	1773	40.3	1.48	12.1	30.0
Prophylactic administration (stages 1-2-3)	840	603	71.8	237	28.2	2.54		

$E = I B + II A$ $A = II B + III + IV$ $D =$ Decrease in per cent $D_A =$ Decrease in per cent related to the number of patients with advanced stages

Table V B Decrease in advanced stages after prophylactic administration of alkoxyglycerols (Stage I A excluded)

$E = I B + II A$ $A = II B + III + IV$

p	No of patients	E		A		E/A	D (%)	D (%)
		n	%	n	%			
Radiotherapy (8-75)	4076	2253	56.0	1773	44.0	1.27	17.9	29.3
Prophylactic administration (stages 1-2-3)	762	525	68.9	237	31.1	2.22		

a numerical evaluation of the shift in stage following prophylactic administration of alkoxyglycerols it is convenient to subdivide the stages into advanced stages A = stage II B and higher and early E = II A and lower. Based upon bimanual examination the referral of patients to group A or E is with a high degree of accuracy while the differentiation between other stages often presents difficulties. The distribution of A and E

($E_1 = I A + I B + II A$ and $E_2 = I B + II A$) for the different groups is given in Table V. The decrease in the advanced stages (D) after prophylactic administration is statistically significant ($P < 0.001$).

The percentage of early and advanced stages is given for each year during 1958-75 (Table VI). No systematic change with time is observed for the years when the patients were treated with radiotherapy but did not receive alkoxyglycerols pro-

Table VI Distribution of early and advanced stages (1958-75)

Year	No of pats	E ₁ I A+I B+II A		A II B+III+IV		E ₁ /A	No of pats	E ₂ I B+II A		A II B+III+IV	
		n	%	n	%			n	%	n	%
1958	428	251	58.6	177	41.4	1.42	419	247	57.8	177	42.2
1959	436	251	57.6	185	42.4	1.36	410	225	54.9	185	45.1
1960	356	201	56.5	155	43.5	1.30	336	181	53.9	155	46.1
1961	401	236	58.9	165	41.1	1.43	379	214	56.5	165	43.5
1962	426	261	61.3	165	38.7	1.58	388	223	57.5	165	42.5
1963	348	225	64.7	123	35.3	1.83	307	184	59.9	123	40.1
1964	381	227	59.6	154	40.4	1.47	337	183	54.3	154	45.7
Period 1 1965	458	333	72.7	125	27.3	2.66	413	288	69.7	125	30.3
1966	309	190	61.5	119	38.5	1.60	266	147	55.3	119	44.7
1967	318	200	62.9	118	37.1	1.69	285	167	58.6	118	41.4
1968	299	171	57.2	128	42.8	1.34	279	151	54.1	128	45.9
1969	290	168	57.9	122	42.1	1.38	259	137	52.9	122	47.1
Period 2 1970-1972	137	92	67.2	45	32.8	2.04	120	75	62.5	45	37.5
1970-1972	142	92	65.0	50	35.0	1.63	125	71	56.8	54	43.2
Period 3 1973-1975	245	178	72.6	67	27.4	2.66	229	165	70.7	67	29.3
1973-1975	266	158	59.4	108	40.6	1.46	236	178	54.2	108	45.8

phylactically. A shift towards earlier stages occurs however for the periods 1 2 3 (prophylactic administration of alkoxyglycerols)

COMMENTS

The regression of tumour growth demonstrated by a change in the quotient between early and advanced stages is observed as a consequence of administration of alkoxyglycerols prior to the radiation treatment. It is of importance to mention that regression is due to a non-toxic natural substance found in the human body (1). Furthermore the incidence of injuries following radiation therapy is markedly reduced by the administration of alkoxyglycerols. Complex injuries (due to radiation injury and tumour growth in combination) are reduced to about 1/3 in a group receiving alkoxyglycerols in prophylactic administration. A more detailed analysis of the effect of alkoxyglycerols on the incidence of injuries is published elsewhere (7).

The regression of tumour growth and the decrease in injuries following radiotherapy influences the survival rate. This dependence will be discussed in a separate paper (6).

It is evident that there are prospects of improving the effect of alkoxyglycerol therapy. Seven days of prophylactic administration of alkoxyglycerols results in a marked reduction of the advanced stages

12-13% (30% if the decrease is related to the number of patients with advanced stages). It would be worthwhile carrying out clinical trials with a patient group receiving prophylactic administration of alkoxyglycerols during 4 weeks. Furthermore some observations indicate that a prolonged administration of alkoxyglycerols after radiotherapy might be of value.

ACKNOWLEDGEMENTS

This work was supported by grants from the Knut and Alice Wallenberg Foundation and from the S. Dagmar Salen Foundation.

We wish to thank Prof. H. L. Kottmeier and Dr. Tjernberg, former and present head of the Department of Gynaecology of Radiumhemmet, Stockholm for their discussions. Our thanks are also due to Prof. H. Jönsson for stimulating criticism and advice.

REFERENCES

1. Brohult A. Alkoxyglycerols and their use in radiation treatment. *Acta Radiol Suppl* 227 1963.
2. Brohult A, Brohult J & Brohult S. Biochemical effects of alkoxyglycerols and their use in radiation therapy. *Acta Chem Scand* 24 730 1970.
3. Brohult A, Brohult J & Brohult S. Effects of alkoxyglycerols on the serum ornithine carbonyl transferase in connection with radiation therapy. *Experientia* 28 146 1972.
4. Brohult A, Brohult J & Brohult S. Effects of radiation and alkoxyglycerol treatment on the

- tion of antibodies after Salmonella vaccination. *Ex* 954 1977
- Brohult A, Brohult J & Brohult S. Effect of alkoxyglycerols on the frequency of injuries following radiation therapy. *Experientia* 29 81 1973
- Brohult A, Brohult J & Brohult S. Alkoxyglycerols in cancer therapy. To be published
- Brohult A, Brohult J, Brohult N & Joelsson I. Effect of alkoxyglycerols on the frequency of injuries following radiation therapy for carcinoma of the uterine cervix. *Acta Obstet Gynecol Scand* 4 441 1977
- Hallgren H & Larsson S. The glyceryl ethers in the liver oils of elasmobranch fish. *J Lipid Res* 3 31 1962
- Hallgren H & Larsson S. The glyceryl ethers in man and cow. *J Lipid Res* 3 39 1967
- Joelsson I. Radiotherapy of carcinoma of the uterine cervix with special regard to external irradiation. *Acta Radiol Suppl* 307 1970

Submitted for publication May 16 1977

Astrid Brohult
Radiumhemmet
Karolinska sjukhuset
Stockholm 60
Sweden

ANNOUNCEMENT

The 6th European Congress of Perinatal Medicine is being held in Vienna, Austria, August 30–September 1, 1978.

Information on the congress as well as programs may be obtained from the secretariat: Interconvention GmbH, Gasse 5, A-1095 Vienna, Austria.

IMMUNOCHEMOTHERAPY IN ADENOCARCINOMA OF THE OVARY

P. K. Kalpaksoglou, G. H. Ioannidou, A. P. Kondyli, S. I. Lekou,
D. Papaconstantinou and A. C. Comminos

*From the Immunobiology Research Centre, Marka Eliadi Maternity Hospital
Athens, Greece*

Abstract. Immunochemotherapy combined with total hysterectomy was used in the treatment of 21 cases of adenocarcinoma of the ovary of which 7 were in stages Ia, 8 in stage III and 6 in stage IV. Another 6 cases had fixation with or without hysterectomy or various other measures of treatment prior to immunochemotherapy. The immunotherapy depends on stimulation of the humoral cellular immunity by a battery of antigens (Tc, D₁-Tc, influenza, mumps). Chemotherapy starts by administration of cyclophosphamide three days after each course of immunotherapy. Each course of immunochemotherapy is followed by a period of 10 days free from any medication, stimulation of the immune mechanism with BCG 10 days after the 4th course of immunochemotherapy. This last course of treatment is repeated up to the end of the first year of immunochemotherapy. The same annual scheme of treatment is constantly repeated. The patients are under constant clinical and laboratory supervision. The cyclophosphamide is discontinued when the WBC falls below 4500 to 4000 per cmm. All patients receive vitamins A, E, C and B complex. All our cases of stages Ia-IIb and III with total hysterectomy without any prior treatment showed a progressive recovery and are living normal lives. The lymphocytes, immunoglobulins and the E rosettes returned in most cases to normal levels. The favourable results and survival rate obtained in cases of cancer without distant metastasis may be attributed to the reduction of the tumour masses by surgery to the administration of immunoprior chemotherapy which seems to diminish the side effects of cyclophosphamide to the administration of cyclophosphamide in small daily doses to avoid myelosuppression and finally to the fact that patients are under continuous protective immunochemotherapy.

In previous studies we have shown that combined immunotherapy and chemotherapy in mice with transplantable myeloma tumour (3) or spontaneous mammary carcinoma (4, 6, 9) decreases the mortality rate. Also immunochemotherapy in carcinoma of the reproductive system of the human female

results in a rapid amelioration of the patient's general condition and a prolonged recovery (1, 7, 8, 9).

The purpose of our present study is to present the results obtained by immunochemotherapy in cases of adenocarcinoma of the ovary stages Ia-IIb, III and IV (FIGO).

MATERIAL AND METHODS

Scheme of treatment. The scheme of treatment is divided into two parts. First, the stimulation of humoral immunity by a battery of antigens in four consecutive courses of treatment and second, the stimulation of cellular immunity by BCG antigen administered orally.

Fig. 1 shows the scheme of treatment applied. The active nonspecific humoral immunization is divided into four consecutive courses. In the first course we administer tetanus antigen (Tc 1 ml S.C.) in the second diphtheria—Tc (D₁-Tc 1 ml S.C.) in the 3rd D₁-Tc and three days later influenza antigen in the 4th course D₁-Tc three days later influenza and after another three days mumps antigen.

Chemotherapy starts three days after each course of immunotherapy with administration of cyclophosphamide in a total dose of 60 mg/kg (stages III and IV) or 30 mg/kg (stages Ia-IIb) per course of treatment divided into daily doses not exceeding 200 mg. A period of 10 days free from any medication is always left between the courses of immunochemotherapy.

The stimulation of cellular immunity starts 10 days after the 4th course of immunochemotherapy. We administer 10 mg of BCG orally every second day up to the total dose of 60 mg. After three days free from medication the patient receives daily 50 mg of cyclophosphamide orally for 10 to 15 days. This last course of treatment is repeated up to the end of the first year of immunochemotherapy with intervals of 10 days free from any medication between each course of treatment. The same annual scheme of treatment is repeated with the only difference that cyclophosphamide is administered orally and in a daily dose of 50 mg for 10 days.

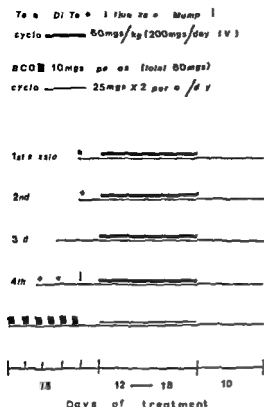


Fig 1 Scheme of immunochemotherapy

During the first four courses of treatment the patients undergo a check up every 5 to 10 days which includes WBC Ht platelet count determination of serum levels of the immunoglobulins G A and M and the NBT positive cells by a modified method (5). Since the beginning of 1975 we have also studied by a micromethod (10) the number of spontaneous E rosettes in the peripheral blood before and again before every second course of immunotherapy.

The administration of cyclophosphamide is discontinued whenever the WBC falls below 4500 to 4000 per mm³. Antibiotics are administered whenever the NBT positive cells exceed the absolute number of 1200 per mm³ or the percentage of 20.

All patients are under continuous vitamins A E B complex and C treatment.

The above scheme of treatment has been combined with total hysterectomy in 21 cases of adenocarcinoma of the ovary of which cases 7 were in stages Ic-IIb 8 in stage III and 6 in stage IV. None had any other prior treatment. We have also treated another 6 cases 3 in stage III (one with prior irradiation and two without total hysterectomy) and 3 in stage IV with various prior schemes of treatment and surgery.

RESULTS

All but one of our cases of stages Ic-IIb and III with hysterectomy showed satisfactory recovery. The

exception is a patient who 77 months after beginning of treatment had partial intestinal obstruction. All cases in stage IV had an initial period of 2 to 4 months of moderate recovery, a relapse and a survival rate not exceeding 7 months.

Fig 2 shows the survival rate of cases of stages Ic-IIb and III. As shown in this figure all cases treated by total hysterectomy showed satisfactory recovery and live normal lives. Two cases of stage III without hysterectomy died, the one 17 months and the other 25 months after treatment started.

Fig 3 shows the serum levels of the immunoglobulins G A and M in IU/ml just before treatment. The immunoglobulin G in normal individuals ranges between 90 and 140 IU/ml, IgA between 100 and 180 IU/ml and IgM between 200 and 240 IU/ml. As shown in Fig 3 cases of stage IV show levels of IgG below 80 IU/ml (3 cases), IgA above 250 IU/ml (one case) and IgM below 100 IU/ml (3 cases). In cases of stages Ic-IIb and III the immunoglobulins remain or return to normal levels during the whole period of treatment.

Fig 4 shows the absolute number and percentage of circulating lymphocytes just before the beginning of non specific humoral immunization (Te₁) and before the beginning of non specific humoral immunization (B C G₁) in cases of stages Ic-IIb and III. The absolute number of lymphocytes shows a drop in all cases. This drop is related to the decrease in the number of circulating lymphocytes.

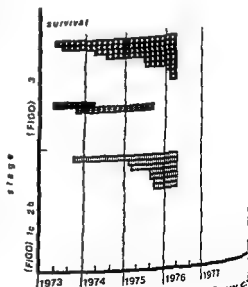
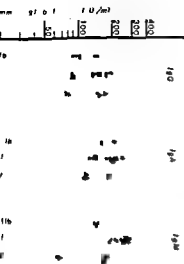


Fig 2 Length of survival of the cases 7 cases of stages Ic-IIb (—) 9 cases stage III with hysterectomy (---) 3 cases stage III without hysterectomy (·····)



Serum levels of the immunoglobulins G, A and M before treatment in cases with hysterectomy and any prior treatment (●) with hysterectomy and irradiation (○) without hysterectomy and without treatment (■) with prior multiple schemes of treatment and surgery (▲)

lymphocytes which ranged between 7000 and 12000/mm³ before treatment (Te₁) and between 6000 and 12000/mm³ before the beginning of BCG. The percentage of lymphocytes increases in most of the cases of stages Ic-IIb and decreases in cases of stage III.

Figure 4 shows the absolute number and the percentage of the spontaneous E rosettes. In normal individuals the absolute number and the percentage of E rosettes range between 100 and 200/mm³ and 7 and 20% respectively. Out of the 15 cases of stages Ic-IIb studied, only two show low absolute numbers and low percentages just before the beginning of immunotherapy (Te₁). In 13 cases the E rosettes show a significant rise before the beginning of BCG. In two they remain unchanged and in one a significant drop is noted. In the latter case the absolute number and the percentage of E rosettes have returned to normal levels two months later. Out of 5 cases of stage III, one showed an absolute number of E rosettes below 100/mm³ and a percentage below 5% before treatment. In four of these cases the absolute number and the percentage of E rosettes returned to normal levels just before the beginning of BCG. In one case two months later.

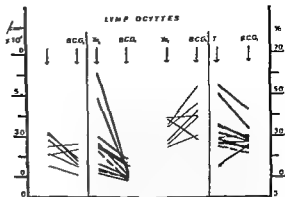


Fig 4 Absolute number and percentage of circulating lymphocytes just before the beginning of non specific humoral immunization (Te₁) and before the beginning of non specific cellular immunization (BCG) cases of stages Ic-IIb (—) cases of stage III with hysterectomy (---) cases of stage III without hysterectomy (· · ·)

DISCUSSION

Since 1973 we have used a scheme of immunotherapy in cases of adenocarcinoma of the ovary.

Immunotherapy preceding chemotherapy seems to diminish the side effects of cyclophosphamide and to protect the patients lymphohaemopoietic tissues. By administering cyclophosphamide in a small daily dose and in consecutive courses of treatment with intervals of at least 10 days free from any medication we provide the period of time necessary for most of the tissues to return to their normal structure and function. We thus try to restrict the

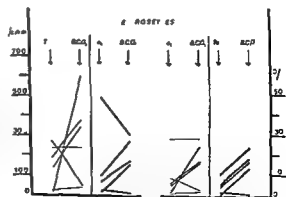


Fig 5 Absolute number and percentage of spontaneous E rosettes cases of stages Ic-IIb (—) cases of stage III with hysterectomy (---) cases of stage III without hysterectomy (· · ·)

uptake of cyclophosphamide to the malignant cells and protect all tissues from irreversible damage

The favourable results obtained in our cases of cancer without distant metastasis may be attributed to the fact that the patients are under continuous protective immunochemotherapy

The effect of the treatment applied depends on the extent of the masses of the malignant cells. We therefore recommend surgery even in advanced cases with the intention to reduce the tumour masses as much as possible

We start our treatment by applying a non specific humoral immunization with the aim to stimulate T cells and macrophages participating in the humoral immune response in order to trigger indirectly the cellular immunity which is very often affected

The study of the spontaneous E rosettes constitutes the proof that our efforts have been successful as in most of our cases the percentage and the absolute number of E rosettes increased after the first or second active non specific humoral immunization

In the cases of stages Ic-IIb the absolute number of circulating lymphocytes shows a drop while their percentage remains either unchanged or is increased. In the cases of stage III both absolute number and percentage of circulating lymphocytes are reduced. As an increase of E rosettes has been observed in most of our cases, we suspect that the reduction of circulating lymphocytes may be associated with their removal from the circulation and their concentration at the tumour rejection area

The levels of the serum immunoglobulins may support the view that immunoglobulin G is rarely elevated while on the contrary high levels of IgA and low levels of IgM often coincide with a poor prognosis. This finding has also been observed in experimental animals (6-9)

Abnormal levels of serum IgG or IgA return very often to the normal values with the progress of the treatment while low levels of IgM rarely do so

In the cases of stage IV the scheme of treatment applied is followed by a short lasting (2 to 4 months) recovery. In this stage of the disease the unsatisfactory results may be attributed to a blockage of the immune system which is unable to respond to any antigen

On the other hand in cases of adenocarcinoma stages Ic-IIb and III the therapeutic effect is excellent followed by a prolonged recovery and a significant increase in the survival rate

ACKNOWLEDGEMENTS

This research work has been supported by National Research Foundation Athens/Greece Grant 1010/ Wellcome Trust London Grant BECH/ENP30

REFERENCES

- 1 Cominos A C, Lekou S I, Apalak K, Kalpaktoglou P. Immunotherapy and therapy in gynecological cancer. *Proceedings 8th Int Congress of Chemotherapy Athens* (G K Daikos) p 400. Hellenic Soc of therapy 1974
- 2 Hudson C N, Levin L, McHardy J E, T A, Curling O M, Crowther M, English Leighton M. Active specific immunotherapy of ovarian cancer. *Lancet* II 877 1976
- 3 Kalpaktoglou P K & Good R A. E pertussis antigen and cyclophosphamide on tumor. *Cancer Res* 30 2841 1971
- 4 Kalpaktoglou P K. The effect of chemical and combined immunotherapy and chemotherapy in mammary carcinoma in C3H mice. In *Proc Chemotherapy Proceedings of the 8th Int. of Chemotherapy Athens 1973* (ed G K Daikos) 477. Hellenic Soc of Chemotherapy 1974
- 5 Kalpaktoglou P K, Padiatellis C P, Sotiriou A & Metaxa C B. Evaluation of tetrazolium test in low birth weight infants. *J Pediatr* 84 441 1974
- 6 Kalpaktoglou P K. Immunotherapy or chemotherapy and chemotherapy applied in mammary carcinoma of C3H mice. 2nd National Congress of Oncology (abstract) 128 1975
- 7 Kalpaktoglou P K, Ioannidou G B, Kostas P, Souli, Marganti K E, Cominos A, Andritsakis G. Treatment of the ovaries with combined immunotherapy and chemotherapy. 2nd National Hellenic Congress of Oncology p 131 Athens 1975
- 8 Kalpaktoglou P K, Kondyli A P, Ioannidou G B, Souli, Marganti K E, Cominos A, Andritsakis G P. Treatment of adenocarcinoma of the ovary with combined immunotherapy and chemotherapy. 9th Int Congress of Chemotherapy 80 (abstract) London 1975
- 9 Kalpaktoglou P K. 1976 Immunotherapy. To be published in Arch Hell Med
- 10 Kalpaktoglou P K, Ioannidou G B & Kostas A P. A micromethod for spontaneous preparation

Submitted for publication Nov 12 1976

A C Cominos
Manka Eladi, Maternity Hospital
Elena Venizelos Foundation
20 Kanari Street
Athens 138
Greece

VAGINAL AGENESIS

An Analysis of Ninety Cases

Carlos Alberto Salvatore and Oriando Lodovici

*From the Gynecology Clinic of the University of Sao Paulo Medical School
Sao Paulo, Brasil*

Abstract This study of 90 cases of vaginal agenesis showed the following results: 50% of the patients sought medical advice when between 16 and 20 years of age; 1% of the patients were not married; amenorrhea was present in 100% of the cases; with hypogastric pains in 10% the uterus was not palpable clinically; in 77.7% of cases 77.7% of the patients had total agenesis; 11.1% partial agenesis and 7.7% had hematometra; though laparotomy 75.5% were found to have normal Fallopian tubes; 92.5% of the cases were histologically normal; laparotomy showed the uterus as solid rudimentary in 5% of the cases and absence of the uterus in 24.4% of the cases; and rudimentary in 46.6%; excretion urography showed renal anomalies in 17.5%; surgical treatment by the McIndoe technique carried out in 90 cases gave 100% satisfactory results; the follow up made between 1 and 5 years in which the long term use of an acrylic mold is recommended in those who do not have sexual intercourse showed permanence of the satisfactory results; 11.3% of the cases of which 83.3% were after one operation only; neovaginal cytology done between one and 6 years showed 72.2% of the cases with acidophily between 5 and 20% with acidophily between 21 and

frequently associated with changes in the urinary system

Surgery is the treatment that offers the best results demonstrated in the practice of neovaginoplasty according to McIndoe (2) but the time of the woman's reproduction life at which it should be carried out is still under discussion. Williams (21) recent technique prepares the neovagina in a situation unfavourable for coitus. Consequently we are still using the McIndoe operation.

In previous works (17, 18) it was shown that according to several authors neovaginoplasty is indicated immediately when there is hematometra and in those patients who have attempted sexual intercourse (1, 2, 5, 13, 15).

We have reported previously 2 patients with hematometra and 18 who have tried to accomplish coitus of whom 16 were married. Generally speaking however neovaginoplasty must be done in cases where the wedding is imminent and according to the case after 17 years of age so as to facilitate the integral development of the personality. If the operation is performed—sometimes before intercourse is likely to occur—there is an increased risk of stenosis of the neovagina.

The methods used in the making of an artificial vagina were reported at length in a previous work in which we studied 42 cases (17) and in which we stressed the efficacy of the McIndoe method.

In the present study we report a total review of the cases we have treated including those quoted previously and those reported by Lodovici (10).

METHOD AND MATERIAL

Our series consists of 90 cases observed at the Gynecologic Clinic of the University of Sao Paulo Medical School. The patients underwent a gynecological examina-

tion. Vaginal agenesis is a legal, social and medical problem of great importance demanding treatment at the right period of the woman's sexual evolution. It is a malformation that sometimes goes unnoticed up to the time of puberty when it is realized that menstruation has failed to appear. Vaginal agenesis is a result of arrested development of the Mullerian ducts; hence its frequent association with uterine aplasia. According to most authors (e.g. 2, 9, 12) the external genital organs have a normal appearance with or without the presence of the hymenal membrane and a small depression where the vaginal opening should be. Vaginal agenesis is easily diagnosed and is seldom connected with the form of intersexuality associated with sex chromosome abnormalities. It is

tion (rectal palpation under narcosis) laparotomy (36 cases) or laparoscopy (54 cases) excretion urography (40 cases) and determination of genetic sex (40 cases)

The operating technique is as follows (19) The patient must be placed in a semigynecological position. We prefer the McIndoe technique for the building of the neovagina using a free skin graft taken from the abdomen. The operation has two fundamental parts: the preparation of the graft followed by construction of the tunnel between the rectum and the urethra and bladder. We usually operate together with a plastic surgeon.

The abdominal stage

After preparing the abdominal skin the plastic surgeon takes a skin layer with the dermatome big enough to cover the acrylic mould. The raw part of the abdomen is protected with gauze and cotton and bandaged. The plastic surgeon then prepares the mounting of the epidermis over the acrylic mould sewing the edges with nylon thread while the gynecologist prepares the neovaginal tunnel.

The vaginal stage

An incision is made with the scalpel transverse to the vestibule or to the retrohymenal fossa between the anus and the urethral meatus. One tries to cut first transversely the bundles of the fibrous perineal nucleus found between the urethra and the anus-rectum. The incision is deepened and two small tunnels are formed on either side of the urethra. These are subsequently united in the mid line. By means of the scalpel or its handle or else with curved tip scissors the urethra and bladder are separated from the rectum up to the point where the base of the peritoneal bladder-rectal sac is reached. In this stage of the procedure we never open the urethra, the bladder or the rectum—an accident which often happens when the surgeon attempts to advance the dissection through the mid line. The tunnel must be large enough to a medium sized acrylic mould. Absolute haemostasis of small vessels is obtained with plain catgut number 00.

According to the case a partial dissection should be done at the sides of the pillars of the elevator ani muscle so as to avoid subsequent stenosis of the vaginal introitus.

After the mould is prepared and haemostasis is confirmed and after the tunnel has been soaked with physiological serum the acrylic mould covered with the epidermis taken from the abdomen is introduced.

The mould is fixed with cloth bandages passing through the mould's screw and attached to the abdominal bandages with sticking plaster.

The patient must remain in bed until the seventh or eighth day when she is allowed to walk and to take out the mould. She must subsequently undergo re dressing every 48 hours the mould being removed and re inserted. She must be instructed concerning the long term hygiene of the neovagina in order to avoid stenosis. Details of the post operative management are to be found in other papers (10, 17).

An acrylic mould is inserted soon after surgery and it remains in place for 8 days. The dressing is changed every

other day and after discharge about the 14th day the patient herself will prepare and apply the dressings, consisting of removing and reinserting the mould and the neovagina over a period of 6 months.

ANALYSIS OF THE MATERIAL

Age (90 cases of vaginal agenesis)

10-15 years 7 cases
16-20 years 46 cases (50%)
21-25 years 24 cases
26-30 years 10 cases
31-35 years 4 cases

Civil status

Unmarried 64 cases (71.1%)
Married 26 cases (28.9%)

Symptoms

Amenorrhea 90 cases (100%)
Impossibility of coitus 22 cases (24.4%)
Hypogastric pain 18 cases (20%)

Gynecological examination (Rectal)

Non palpable uterus 70 cases (77.7%)
Rudimentary palpable uterus 14 cases
Uterus increased in volume 4 cases
unrecognizable ovaries 80 cases (88.8%)
Palpable ovaries 8 cases

Diagnosis

Total agenesis 70 cases (77.7%)
Partial agenesis 10 cases (11.1%)
Male pseudo-hermaphroditism 3 cases
Total agenesis plus haematometra 3 cases (7.7%)
Partial agenesis haematometra and haema-
2 cases (7.7%)
Total agenesis haematometra and haema-
2 cases (7.7%)

Internal genital organs observed through Laparoscopy (54 cases) and Laparotomy (36 cases)

(a) Ovaries

Normal 88 cases (75.5%)
Polycystic 6 cases
Hypoplastic 12 cases
Rudimentary 4 cases

(b) Histology of the gonads (biopsy) (40 cases)

Normal ovaries 37 cases (92.5%)
Rudimentary testicles 3 cases

(c) Uterus

Hypoplastic 2 cases
Increased in volume (haematometra) 4 cases
Solid rudimentary 30 cases (55.5%)
Double uterus 1 case
Bicornuate uterus 3 cases
Hemi uterus 4 cases
Two rudimentary uteri 4 cases
Lack of uterus 22 cases (24.4%)

(d) Tubes

Normal 29 cases (32.2%)



Fig 1 Collin speculum in the neovagina

oplasty 6 cases
rudimentary (long) 42 cases (46.6%)
rudimentary tube 2 cases
k of tubes 10 cases
matosalphinx 1 case

retrograde urography (40 cases)
ters and normal kidneys 33 cases (82.5%)
ible pelvis-calices 3 cases
k of left kidney 4 cases

etic sex (40 cases)
ence of the female sexual chromatin 36 cases
k of the female sexual chromatin (male) 4 cases

atment (90 cases)
al neovagina 80 cases (88.8%)
al neovagina 10 cases
vagina and draining of the haematometra through the
agina 3 cases
al neovagina and draining of the haematometra and
ematocolpos (through the vagina) 2 cases
vagina and draining of the haematometra through the
domen (hysterotomy) 1 case
vagina and draining of the haematometra and
ematosalphinx (through the abdomen) 1 case
vagina and clitoris amputation 2 cases

dlis (90 cases)
d graft integration and good vaginal permeability 75
cases (83.3%)
inal stenosis (fibrous ring) (2 re-operated with
atisfactory results) 9 cases
al disintegration of the graft due to a haematoma (all
-operated with satisfactory results) 6 cases
il satisfactory results including the 5 who were re-
perated 81 cases (90.0%)

Follow up (between 1 and 6 years) (70 cases)

Good results (penetration of the Collin speculum
medium sized) (Figs 1-2) 64 cases (91.3%)

Partial stenosis 3 cases

With normal sexual relations 54 cases

With dyspareunia 4 cases

With normal menses 4 cases

Pregnancies (caesarean) 2 cases

Cytology of neovagina (25 cases)
(between 1-6) (Fig 3)

From 5 to 20% of acidophil cells 18 cases (72.0%)

From 21 to 40% of acidophil cells 7 cases (28.0%)

Biopsy of the neovagina showed atrophic epidermis
(Fig 4)

COMMENTARY

As may be observed from an analysis of the cases most patients sought advice in the age range 16 to 20 years (50% of the cases) coinciding with a wait of 3-5 years for the onset of the menarche. As a matter of fact primary amenorrhea was the predominant symptom (100% of the cases). Hypogastric pains occurred in 20% of the cases and attempts at sexual intercourse among those who had tried had been unsuccessful in around 24.4% of the cases.

Of the cases examined 88.9% of the patients were married and had serious problems just after the marriage. Because of such problems most authors think that the ideal age for the making of the neovagina is before the marriage. As we have stated



Fig. 2 Collin speculum in the neovagina (cul-de sac)

in an earlier work (18) libido was present in 79.1% of the 49 cases previously studied.

The diagnosis of vaginal agenesis is an easy one. The gynecological examination by rectal palpation allows the non-identification of the uterus in 77.5% of the cases. Moreover, 77.7% of our 90 cases were of total vaginal agenesis and 11.1% of partial agenesis. There was haematometra in 7 cases (7.7%).

The exploration of the internal genitalia by means of laparoscopy and laparotomy is unavoidable, but

shows that the ovaries are normal in 75.5% of cases. In 3 of the cases (pseudo hermaphrodite) the biopsy revealed rudimentary testicles confirmed by histological study. The psychological analysis in these cases was more important than analysis of the sexual chromatin—hence the indication for a neovagina.

A solid rudimentary uterus was found in 55.5% of the cases, lack of uterus in 24.4% and functional uterus with haematometra in only 7 cases (7.7%). These results agree with those of Counsellor (16).



Fig. 3 Acidophil cells of the neovagina

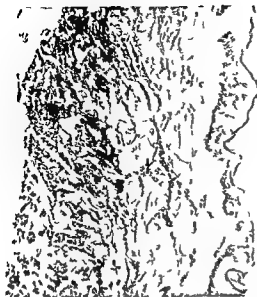


Fig 4 Atrophic epidermis in the neovagina 3 years after the operation

6) of Bryan et al (2) (4 in a 100) Jackson (7) (5 in 128) Cordier (4) (1 in 23) Lodovici (10) (1 in 16) and Jeffloate (8). These findings indicate its existence in 10% of cases. Normal tubes were in the same way found in only 32.2% of the cases.

The practice of excretion urography is an important one as was shown by us and several other authors. The frequency of malformation of the urinary system is very high in cases of vaginal agenesis. Thus the absence of one of the kidneys (4 cases) and double pelvis-calices (2 cases) was observed in 17.5% of the cases. In a previous work in analysis of 21 cases 15 (71.4%) were normal and the rest (28.6%) showed anomalies of the urinary system as represented by the lack of one of the kidneys, dilation of the ureters, of pelvis, double ureters, anomalous implantations, rotation, displacement of one of the kidneys, or dislocated kidney, thus confirming the reports of others (5, 7).

In the experience of many authors (5, 7, 9, 10, 13, 17, 18) the choice of treatment is the making of the neovagina by the McIndoe technique—McIndoe having in 1950–59 established the basic principles for the formation of the neovagina from skin grafts.

Eighty complete neovaginas were made (88.8%) and 10 partial ones, i.e. the vagina was completed in these cases where the agenesis was partial. In two of these cases there was agenesis of the exterior half

of the vagina, the inner half being full of menstrual blood (haematocolpos). The remaining 7 cases showed the presence of a vagina in its exterior third. In the 6 patients who had a functioning uterus perforated moulds were used.

As we have stated in a previous work (18) we recommend the use of an acrylic mould by the patient until the beginning of regular sexual activity, returning to its use in cases where a long interruption occurs.

The results of the McIndoe technique are good as graft integration occurred in 81.3% of the cases. If we add 6 cases where the integration was partial but reoperation was necessary, we achieved 90% of optimum graft integration and optimum conditions of permeability. We observed the presence of a fibrous ring in only 3 of the 90 cases. Page & Owley (14) claim 81% good results.

The late follow-up observed between one and six years showed 91.3% satisfactory results in 70 cases, of which 54 had normal sexual relations, two had become pregnant and undergone caesarian section. Only 6 cases showed a small vaginal stenosis and 4 complained of dyspareunia. We have not so far found enterocele.

In our experience, therefore, the McIndoe technique for the making of an artificial vagina is the one offering the best results and the long-term use of an acrylic mould guarantees the late success of the operation.

stenosis of the introitus occurred in three patients. Our best results were obtained with ambulant dilatation.

Can women also conceive if they have their uterus? Four patients had an uterus present. In two the cervix was absent and the uterus was removed early in the operation. We sewed the two other uteri into the neovagina. The women had regular menstruation but they did not conceive. After six years we removed the uterus in one of them because of endometriosis.

The same technique can also be applied at the end of Wertheim's operation when the greater part of the vagina is removed and if the patient is young and wishes it. The purpose is the same as in the cases of congenital aplasia of vagina to make sexual intercourse possible and in this way to alleviate the physical and psychological deficiency of these women.

The reconstruction of the vagina after Wertheim's operation consists in lengthening the vagina which remains after the operation with a short segment of the colon that we resect from the sigmoid. The technique of this operation is accurately described by L. Kos in his thesis (6).

For reconstruction after the Wertheim operation only a 5 to 6 cm length of sigmoid is sufficient. It is easily displaced downwards to the level of the remaining vagina without having to resect any sigmoid artery. This has several advantages: the neovagina is well supplied with blood and there is no risk of necrosis. As we do not lose time with ligating the sigmoid arteries the reconstruction only takes half an hour at the most.

We form the neovagina by closing the resected sigmoid segment at one end and bringing the other end near the hinder part of vagina in order to perform the colovaginal anastomosis. The cupola of the neovagina can be either the proximal or distal end of the resected segment. It does not matter in which direction peristaltic action occurs. As a matter of fact we perform the anastomosis with that end of the resected sigmoid that can be most easily brought to the hinder part of vagina. However this depends on the anatomy of sigmoidal arteries as well as on origin of the mesosigmoid.

In most cases the lumen of the hinder part of vagina and the sigmoid are not of the same size: usually the lumen of the sigmoid is narrower. It must be enlarged before suturing the colovaginal anastomosis. This is performed by splitting it along the taenia for 2 cm or more if necessary in order to make both lumina approximately the same size. By this simple act the formation of stenoses at the colovaginal anastomosis is prevented. Our experience has also been that silk is most appropriate for suturing the colovaginal anastomosis. The stitches must not be made too closely: three stitches in front and three behind are sufficient.

In reconstructions performed at the same time as the Wertheim operation we had no deaths. We had one rectovaginal fistula which closed spontaneously after tem-

porary colostomy and one abscess which drained after the colovaginal anastomosis.

We advise against reconstruction of the vagina with sigmoid colon after Wertheim's operation in patients who have been irradiated before the operation in early women in cases of inflammation in the pelvis as well as in the cases where the sigmoid is short.

None of the patients complained after discharge. After the reconstruction of vagina there was no stress incontinence of urine with any of the patients. While it is well known that many patients have this difficulty after gynecological operations. There could be an explanation for this in the fact that the neovagina with its mesenteries gives to the bladder support similar to that given by uterus and the vagina before the operation.

REFERENCES

- 1 Aleksandrov M S. Obrazovanie iskusstvennoy vaginali iz sigmoidovidnoi kisiki. Medgiz 1955.
- 2 Baldwin J F. The formation of an artificial vagina by intestinal transplantation. Ann Surg 40: 398 1905.
- 3 Brindeau A, Lantuejoul P & Hubert L. Création d'un vagin artificiel à l'aide des membranes muqueuses d'un œuf à terme. Gynecol Obstet 45: 417 1946.
- 4 Cigovski E E. Odnorukavni metod obrazovanja kusstvenoga vaginali iz sigmoidovidnoi kisiki. Akte simpozija ginekologija 5: 48 1955.
- 5 Graves V P. Method of constructing an artificial vagina. Surg Clin North Am 1: 611-614 1911.
- 6 Kos L. Rekonstrukcija vagine po Wertheimu. Schauta-Amreichovi operaciji s segmentom sigmoidnog kolona. Disertacija Medicinska fakulteta Ljubljana 1976.
- 7 Novak F. Težnja k kirurški ginekologiji. Folia clin Padova 1973.
- 8 McIndoe A H & Bannister J B. An operation for the cure of congenital absence of the vagina. J Obstet Gynecol Br Emp 45: 490-494 1938.
- 9 Ruge E. Ersatz der Vagina durch Flexura sigmoidea. Laparotomie. Disch Med Wochenschr 40(11): 1914.
- 10 Schmid H H. Scheidenbildung aus dem Dickdarm. G Fischer Verlag, Jena 1946.
- 11 Shirodkar V N. The problem of artificial vagina. Tendances actuelles en gynécologie et obstétrique, vol 1 p 573. Beauchemin, Montréal 1978.

Submitted for publication Nov. 22 1976

Franc Novak
Department of Obstetrics and Gynecology
University of Ljubljana
Yugoslavia

HAEMODYNAMIC EFFECTS OF OXYTOCIN (SYNTOCINON®) AND METHYL ERGOMETRINE (METHERGIN®) ON THE SYSTEMIC AND PULMONARY CIRCULATIONS OF PREGNANT ANAESTHETIZED WOMEN

N J Secher P Arnsbo and L Wallin

From the Department of Gynecology & Obstetrics the Department of Clinical Physiology and the Department of Anaesthetics Odense University Hospital Odense Denmark

Abstract The haemodynamic effects of oxytocin (Syntocinon®) and methyl ergometrin (Methergin®) were studied in 9 healthy females in the first trimester of pregnancy. The patients were anaesthetized with sodium thiopental, pethidine and pancuronium bromide and ventilated on a Manley respirator. 10 i.u. oxytocin given in 4 bolus brought about a fall in femoral arterial pressure of 40%, systemic resistance 59% and pulmonary resistance 44% 30 sec after injection. However the heart rate increased 31% and stroke volume 17% so that the cardiac output increased by 34%. The pulmonary arterial pressure and wedge pressure were increased by 33% and 27% respectively 150 sec after injection. No changes were seen in the haemodynamic parameters during infusion of 100 mU oxytocin for 10 min. 0.2 mg Methergin brought about an increase in the femoral arterial pressure of 17%, pulmonary arterial pressure 27% and wedge pressure 31% with no changes in the other measured parameters. The use of oxytocic drugs in patients with compromised circulation is discussed.

pare the changes with those found in the systemic circulation.

The patients were examined in the first trimester of pregnancy in order to exclude the circulatory changes caused by autotransfusion from the uterus during contraction at term.

MATERIAL AND METHODS

The aim of the study and the experimental procedure was explained in detail to the patients. At the same time it was stressed that there would be no therapeutic benefit from participating in the study. Thereafter all the patients consented to take part. The material consists of 9 healthy women age 20-37 years (mean 28) referred to the clinic for abortion in the 10th to 17th weeks of gestation. The patients were anaesthetized with sodium thiopental, pethidine and pancuronium bromide. They were intubated and ventilated on a Manley respirator adjusted according to the Radford nomogram. Ventilation was carried out with N₂O-O₂ in a ratio of two to one so as to obtain as stable and reproducible circulation as possible. The ECG was registered continuously and the pressure in the femoral artery measured continuously through an indwelling catheter. A Swan-Ganz flow directed thermodilution catheter (93A 118 7F) was introduced into the pulmonary artery through an antecubital vein. The pulmonary artery pressure, the pulmonary wedge pressure and the femoral pressure were measured using pressure transducers (Elema Schonander type EMT 35). The results were registered on a recorder (Mingograph 81 Elema Schonander). The transducers used were calibrated before and after each series of measurements. The output of the pulmonary and wedge pressure transducer were averaged electrically to obtain the mean pressure. All the measurements were performed with the patient in the supine position and the level of the zero reference point of the pressure recordings was defined as the midaxillary line. The thermistor in the Swan-Ganz catheter was connected to a cardiac output computer (Edwards 9510) the output of which was registered on a recorder (Servogor RE 570). Any abnormal curves were disregarded. The

cardiovascular effect of oxytocin given intravenously in the dosage required for the induction of labour have been considered as minimal since the induction of synthetic oxytocin (5, 10, 16, 22). In contrast to this several studies of pregnant women in a bolus injection of intravenous oxytocin have demonstrated hypotension with a concomitant increase in cardiac output (10, 14, 22). Anaesthesia does not appear to influence the circulatory effect of oxytocin to any great extent (2, 5). Ergometrine has been shown to increase the systemic and the central venous pressures (6, 9, 10, 23, 24) but its synthetic analogue methyl ergometrine (Methergin) has been reported to produce much less hypertension (8, 21). The purpose of this investigation was to study the cardiovascular effects of oxytocin (Syntocinon) and methyl ergometrine (Methergin) on the pulmonary circulation and further to com-

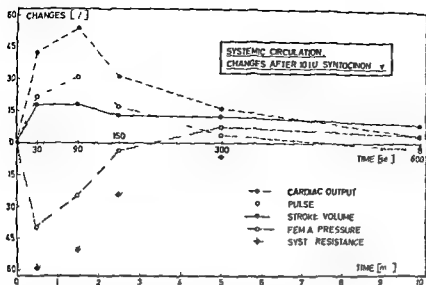


Fig 1
hemodynamic parameters in systemic circulation with after 10 i.u. oxytocin (Syntocinon®)

trans-thoracic electrical impedance (Z_a) which is a function of the thoracic fluid volume (17) was measured by a Minnesota impedance cardiograph model 304A designed by Kubicek et al (15). Two aluminium strip electrodes were placed around the neck separated as widely as possible and two electrodes were placed around the abdomen. The upper at the level of the xiphoid process and the other electrode 5 cm below. The outer electrodes were provided with an electrical field from a constant sinusoidal current oscillator (100 kHz 4 mA).

The voltage between the two inner electrodes was measured and the impedance Z was displayed on a digital meter.

PROCEDURE OF THE INVESTIGATION

Control measurements of ECG, blood pressures and impedance and at least 5 cardiac output measurements were made after completion of suction abortion.

Two of the patients then received 5 i.u. oxytocin. Patients received 10 i.u. oxytocin as a bolus through a peripheral arm vein. The remaining patients received a constant infusion of 100 mU/min of oxytocin over a period of 10 min. The cardiac output and thoracic impedance were measured after 30 sec and after about 90, 150, 300 and 600 sec respectively. Pressure in the femoral artery was measured continuously.

Table 1 The maximal effect of oxytocin (Syntocinon®) on the haemodynamic parameters of the systemic circulation

Patient	Fem art press (mmHg)		Systemic resistance (dyn sec cm ⁻⁵)		Stroke volume (ml)		Pulse (min ⁻¹)		Cardiac output (ml)
	Before	After 30 sec	Before	After 30 sec	Before	After 90 sec	Before	After 90 sec	Before
1 5 i.u. of Syntocinon	83	40	1 254	477	64	74	83	94	5 706
2	83	60	1 358	698	58	73	85	91	4 888
1 10 i.u. of Syntocinon	90	53	1 378	529	77	82	68	97	5 255
2	73	60	1 150	699	67	76	76	79	5 080
3	95	56	1 617	613	59	80	79	111	4 700
4	98	58	1 421	640	69	90	80	107	5 518
5	93	70	1 879	565	71	72	65	97	3 960
6	89	46	1 123	498	75	83	84	107	6 340
7	83	46	1 377	581	67	78	77	97	4 813
8	89	46	1 323	532	69	82	78	105	5 380
9	79	39	1 036	401	80	89	76	111	6 100
Mean	87.7	57.7	1 367	562	69.3	81.3	75.3	98.6	5 236
Mean ± S.D.	8.0	9.5	261	87	7.0	5.8	6.0	10.7	773

$p < 0.001$

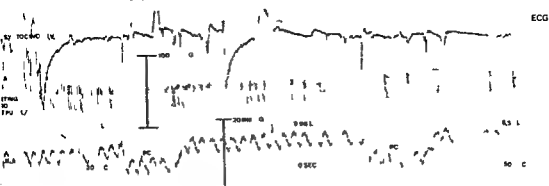


Fig. 2 The effect of 10 i.u. of oxytocin given as a bolus intravenous on the pulmonary arterial pressure (PULM)

A) wedge pressure (PCV) and femoral arterial pressure (FEM A) on one subject

was the pulmonary arterial pressure although the recording was interrupted by measurements of the wedge pressure. After at least 10 min all the patients received an intravenous bolus injection of 10 i.u. of oxytocin. After circulation had again become stabilized all the patients received 0.2 mg methyl ergometrine (Methergin) intravenously and measurement of the haemodynamic parameters was repeated for a period of about 10 min.

RESULTS

The effect of 10 i.u. of oxytocin given as a bolus intravenous injection on the haemodynamic parameters

of the systemic circulation in 9 subjects are shown in Table I and Fig. 1. A typical example is shown in Fig. 2. The decrease in the femoral arterial pressure was most pronounced after 30–40 sec with a decrease of 40% below the control values; the blood pressure returned to control levels after 150 sec. This hypotension was followed by a concomitant increase in stroke volume as well as in heart rate. The heart rate increased approximately 10 sec after the decrease in the arterial pressure and a maximal increase of 31% was obtained after about 90 sec. The stroke volume increase followed

Table II The maximal effect of oxytocin (Syntocinon[®]) on the haemodynamic parameters of the pulmonary circulation

Patient no.	Pulmonary resistance (dyn sec cm ⁻⁵)		Pulm. art. press (mmHg)		Wedge press (mmHg)	
	Before	After 30 sec	Before	After 150 sec	Before	After 150 sec
10 i.u. of Syntocinon	35	24	11.2	14.6	8.9	11.1
	87	47	11.7	13.3	6.2	7.6
10 i.u. of Syntocinon	77	—	13.8	17.5	9.1	—
	90	51	13.3	13.8	7.6	8.9
	31	11	11.0	14.2	9.2	12.3
	59	37	9.3	12.8	5.7	6.0
	89	18	14.4	18.0	10.0	1.8
	71	100	11.6	16.8	6.0	8.8
	65	74	17.1	16.0	8.7	10.7
	54	50	9.6	14.6	6.0	8.9
	53	9	9.4	14.9	5.4	9.2
Mean	64.9	36.6	11.6	15.4	7.4	9.7
SD	18.5	18.1	7.0	1.8	1.8	2.7

*0.01

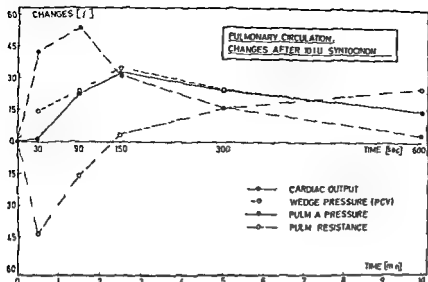


Fig 3 Percentage change in hemodynamic parameters of pulmonary circulation 30 sec after 10 IU oxytocin (Sigma non®)

the change in heart rate and maximal values of 17% of control values were recorded after 90 sec. The cardiac output increased by 54% of the control value after 90 sec and a maximal decrease of 50% in peripheral resistance was observed after 30 sec. The changes in systemic haemodynamics after 5 IU of oxytocin were almost as pronounced as after 10 IU (Table I). The effects of 10 IU of oxytocin given as a bolus intravenous injection on the haemodynamic parameters of the pulmonary circulation in 9 subjects are seen in Table II and Fig 3.

The most striking observation was an increase in pulmonary arterial pressure: this reached maximal values of 33% after 150 sec at which time the

cardiac output was still elevated but the pressure in the systemic circulation was almost normal. The increase in the pulmonary arterial pressure was followed by a concomitant increase of 35% in wedge pressure after 150 sec. After 10 min the pulmonary and wedge pressures were still slightly but significantly elevated. The pulmonary resistance decreased by a maximum of 44% after 30 sec. Pulmonary resistance returned to normal after 30 sec. The changes in the pulmonary haemodynamics after 5 IU of oxytocin were almost as pronounced as those after 10 IU (Table II). Five patients received a second bolus injection of 10 IU of oxytocin and tachyphylaxis was demonstrated by a

Table III The maximal effect of 0.2 mg methyl ergometrine (Methergin®) on the blood pressure in the systemic and pulmonary circulation

Patient no	Fem art press (mmHg)		Pulm art press (mmHg)		Wedge press (mmHg)	
	Before	After	Before	After	Before	After
1 0.2 mg of Methergin	100	120	14.2	17.5	9.8	13.5
2	80	100	14.2	17.8	11.5	10.8
3	96	106	13.5	17.3	9.5	11.0
4	100	116	17.5	15.0	7.9	9.9
5	95	97	16.0	18.4	6.9	10.9
6	88	91	17.7	15.7	6.9	10.9
7	88	94	12.5	18.4	9.8	11.9
8	96	112	10.7	16.4	6.8	11.0
9	83	84	11.7	13.7	8.0	9.8
Mean	91.8	102.2	13.1	16.6	8.8	11.4
Mean \pm S.D.	7.3	12.1	1.6	1.7	1.6	1.3

$p < 0.01$

$p < 0.05$

nounced change in the haemodynamics than after the first injection (heart rate 37% stroke volume cardiac output 42% systemic arterial pressure 90% pulmonary arterial pressure 52% and wedge pressure 64% of the maximal values after the first injection)

No changes were seen in the pulmonary nor the systemic circulation after intravenous infusion of 80 mU of oxytocin for ten min

A small increase in the pulmonary arterial and wedge pressure of 27% and 31% respectively with a slight increase of 11% in the systemic blood pressure were found after 0.2 mg methyl ergometrine. These values reached maximal values after 5 to 10 min. No changes were found in cardiac output and heart rate.

The hypertension persisted throughout the period of observation and the mean maximal pressures in the systemic and pulmonary circulation are given in Table III.

A fall in thoracic impedance of 0.19 ohm was found after 0.2 mg methyl ergometrine intravenously, but no change was found after the administration of oxytocin. Apart from a few extrasystoles probably caused by manipulation of the Swan Ganz catheter there were no abnormalities in the ECG during the administration of oxytocin and methyl ergometrine.

DISCUSSION

After an intravenous bolus injection of oxytocin a decrease in systemic arterial pressure followed by a concomitant increase in cardiac output have been reported in several studies (2, 4, 14, 22). In our study of young healthy females in the first trimester of pregnancy 100 mU of oxytocin decreased the mean blood pressure by 40% with concomitant increases in cardiac output and heart rate of 54% and 31% respectively. Nakano & Fisher (18) found in a study that oxytocin increased the myocardial contractile force. This effect could be caused by oxytocin per se or it could represent a reflex response due to acute vasodilatation.

Our findings of an increased heart rate and stroke volume which appeared later than the peripheral vasodilatation indicate that a reflex sympathetic stimulation is the most likely explanation.

After administration of pitocin Woodbury et al found an increased pressure in the jugular vein with a nonsignificant increase in central venous pressure. This was found by Williams et al (23) after

administration of 10 i.u. oxytocin post partum. We have not measured the central venous pressure but the pronounced increase in cardiac output indicates an increased venous return to the heart. Oxytocin has a peripheral vasodilatory effect on the vascular bed in the human extremities (11, 13). The increased venous return could not be caused by emptying of the vessels of the contracting uterus as the patient was only in the first trimester of pregnancy. The increased blood return is probably caused by visceral vasoconstriction as was also demonstrated in female rats following direct microscopic examination (1). A concomitant factor could be an increased sympathetic drive on the heart with the increase in cardiac output.

Only a few studies have been carried out on the effect of oxytocin on the pulmonary circulation. Intravenous injection of 1 i.u. oxytocin did not change pulmonary arterial pressure nor pulmonary vascular resistance in man (19). However it was found that continued infusion of high dosages of 10–15 i.u. oxytocin over a 30 min period caused a slight increase in pulmonary arterial pressure on average of 1.6 mmHg. In this study (19) 9 of 15 male patients showed an increase in pulmonary arterial pressure. This increase in pulmonary pressure was not associated with any change in pulmonary wedge pressure or pulmonary vascular resistance. We found no change in the pulmonary arterial and wedge pressures during infusion of 80 mU of oxytocin per minute for 10 min. After intravenous injection of 10 i.u. of oxytocin we have found an increase in pulmonary arterial and wedge pressures in all patients; these reached maximal values of +33% and +35% after 150 sec when the systemic blood pressure was almost normal but with a cardiac output that was still elevated. This increase in pulmonary pressure was preceded by a transient decrease in pulmonary vascular resistance. This increase in pulmonary and wedge pressures could be caused by the increase in cardiac output per se as seen during exercise (7).

Many investigators have reported that the circulatory effect of repeated doses of oxytocin are weaker than those of the initial doses (4, 12, 24). In our study the cardiovascular effect of the repeated bolus dosage of 10 i.u. of oxytocin was less pronounced than that following the initial dose. This tachyphylaxis has been suggested as being due to a change in the response of the blood vessels to the direct actions of oxytocin (13).

18884 BT ROBERT HELIC LIBRARY

The effect of oxytocin on the ECG has been studied in several investigations (12-16). Berquist (3) found not only alteration of the T wave but also occasional depression of the ST segment in post partum women. Katz (12) found as we did that there were no essential ECG changes in anaesthetized patients.

Oxytocin is frequently given as a bolus injection after delivery and during suction abortion although it has been stressed that oxytocin if it is to be used intravenously should be administered as a diluted intravenous infusion (10-22). Although the haemodynamic changes after an intravenous bolus injection of oxytocin were transient the haemodynamic changes could be dangerous in patients with a circulation already compromised by hypovolaemia and heart disease with a fixed cardiac output, valvular stenosis or right to left shunts.

It has been demonstrated that methyl ergometrine raises the central venous pressure and reduces peripheral venous compliance in healthy volunteers (6-9, 23). Our results confirmed that a slight elevation in systemic blood pressure occurs after methyl ergometrine (8, 21). We found in percentage a more pronounced increase in pulmonary arterial and wedge pressures after methyl ergometrine. The concomitant fall in thoracic impedance of 0.19 ohm indicates an increase in thoracic fluid volume. As methyl ergometrine constricts the blood vessels the fall in thoracic impedance would suggest an increase in the interstitial fluid lung volume. This finding together with the reduced compliance of the venous reservoir after methyl ergometrine and its effect together with an increased blood volume after uterine contraction may be an important factor in the precipitation of pulmonary oedema and cardiac failure in patients with impaired cardiac function. However the risk of post partum haemorrhage is increased if no oxytocic drug is used. Therefore when treating patients with cardiac disease it is necessary to weigh the risk of pulmonary oedema and congestive heart failure following ergometrine with the possibility of a massive post partum haemorrhage if it is not administered.

Prostaglandins have been used in recent years for the induction of labour and midtrimester abortion. We have shown that prostaglandin $F_{2\alpha}$ markedly increases the pulmonary as well as the wedge pressures (20). In this study no cardiovascular changes were found during the infusion of 80 mU of oxytocin per minute. Therefore oxytocin given as a di-

lute intravenous infusion should be the drug of choice in patients with cardiac disease.

REFERENCES

- Altura B M & Hershey S G Pharmacological neurohypophyseal hormones and their analogues in the terminal vascular bed *Angiology* 18 478 1967
- Andersen T W, De Pauda C B, Stenger J, Prystowsky H Cardiovascular effects of intravenous injection of synthetic oxytocin during elective Cesarean section *Clin Pharmacol Ther* 1965
- Berquist J R & Kaiser I H Cardiovascular effects of intravenous syntocinon *Obstet Gynecol* 1959
- Bieniarz J Extra uterine actions of oxytocin Oxytocin (ed R Caldeyro-Barcia & H Hellel) Pergamon Press London 1961
- Bonica A J J Principles and practice of obstetric analgesia and anesthesia 316 F A Davis Philadelphia 1977
- Brooke O G & Robinson B F Effects of ergotamine and ergometrine on forearm venous compliance in man *Br Med J* 139 1970
- Ekelund L G & Holmgren A Central haemodynamics during exercises Suppl 1 to *Circulation Research* vol XX and XXI pp 133 1967
- Fitzgerald W J Methergine A study of its effect on blood pressure *Obstet Gynecol* 167 196
- Greenhalf J O & Evans M J E Effects of ergometrine on the central venous pressure at third stage of labour *J Obstet Gynaecol Br C* 70 1066 1970
- Hendricks C H & Brenner W E Cardiovascular effects of oxytocic drugs used post partum *Obstet Gynecol* 108 751 1970
- Johnstone M The cardiovascular effects of oxytocin drugs *Br J Anaesth* 44 326 1972
- Katz R L Antiarrhythmic and cardiovascular effects of synthetic oxytocin *Anesthesiol* 25 643 1964
- Kitchin A H, Konzett H & Pickford M Comparison of effects of valyl¹-oxytocin and syntocinon on the cardiovascular system of man *Br J Pharmacol* 14 567 1959
- Kitchin H, Lloyd S M & Pickford M Sympathetic actions of oxytocin on the cardiovascular system of man *Clin Sci* 18 399 1959
- Kubicek W G, Karnegis J N, Patterson J, Witsoe D A & Mattson R H Developmental evaluation of an impedance cardiac output monitor *Aerospace Med* 37 1208 1966
- Lipton B, Hershey S G & Baez S Cardiovascular effects of oxytocin with anesthetic agents *JAMA* 197 1962
- Luepker R V, Michael J R & Warshaw J Transthoracic electrical impedance: Quantitative evaluation of a non invasive measure of thoracic volume *Am Heart J* 85 83 1973
- Nakano J & Fisher R D Studies on the

- diavascular effects of synthetic oxytocin *J Pharmacol Exper Therap* **142** 206 1963
- Ribot S Abramowitz S Green H Small III J & Schwartz I Cardiovascular effects of oxytocin and comparison with related polypeptides *Am J Med Sci* **248** 95 1964
- Secher N J & Andersen L H Changes in the pattern of regional pulmonary blood flow after $\text{PGF}_{2\alpha}$ infusion in pregnant women *Cardiovasc Res* **11** 26 1976
- Schade F F Methergine A study of its vasomotor properties *Am J Obstet Gynecol* **61** 188 1951
- Weis F R Markello R Mo B & Bochechio P Cardiovascular effects of oxytocin *Obstet Gynecol* **46** 211 1975
- Williams C V Johnson A & Ledward R A comparison of central venous pressure changes in the third stage of labour following oxytocic drugs and diazepam *J Obstet Gynaecol Br Comm* **18** 596 1974
- 24 Woodbury E A Hamilton W F Abreu B E Torpin R & Fried P H Effects of posterior pituitary extract oxytocin (Pitocin) and ergonovine hydracrylate (Ergotrate) on uterine arterial venous and maternal effective placental arterial pressures in pregnant humans *J Pharmacol Exp Therap* **80** 256 1944
- 25 Woodbury R A Hamilton W F Volpitto P P Abreu E E & Harper H T Cardiac and blood pressure effects of pitocin (oxytocin) in man *J Pharmacol Exp Therap* **81** 95 1944

Submitted for publication Febr 15 1977

✉ J Secher
Dept of Obstetrics and Gynecology YA
Rigshospitalet
Blegdamsvej 9
DK 2100 Copenhagen Denmark

There is no substitute for quality



AB STILLE-WERNER

Box 43051 S-100 72 STOCKHOLM SWEDEN

A/S Stille Werner ■ F Riche vej 103 DK 2000 København DANMARK

OY Stille AB Nervanderinkatu 5 D SF 001 00 Helsinki 10 FINLAND

Stille A ■ Postboks 61 Leirdal Oslo 10 NORGE

Stille AG Postfach CH-8038 Zurich SCHWEIZ

Stille Werner (U.K.) Ltd 24 York Road Maldenhead Berkshire SL6 1SF ENGLAND

Stille GmbH Zulpicher Platz 7 D 5000 Köln 1 BRD

MANUFACTURING AND SALE OF surgical instruments operating
tables medical technical equipments disposables specialities

STILLE

10 years

TRANSFER OF ^{51}Cr PLATELETS AND $^{51}\text{CHROMIUM}$ IONS ACROSS THE TERM RHESUS MONKEY PLACENTA

Henk C. S. Wallenburg, Piet H. van Kessel and Anneke Brand

From the Departments of Obstetrics and Gynecology AZR Dijkzigt Erasmus Universiteit Rotterdam and Immunohematology Rijksuniversiteit Leiden The Netherlands

Abstract Determination of platelet life span could provide useful information on platelet kinetics in pregnant women. A usual procedure involves injection of ^{51}Cr labeled platelets into the maternal circulation. Data on possible transfer of ^{51}Cr platelets and ^{51}Cr ions across the placenta is necessary to estimate the radiation dose delivered to the fetus. Since such data cannot be obtained in man the rhesus monkey was chosen as an experimental model. In an acute animal study ^{51}Cr labeled maternal platelets with an activity of approximately $20\ \mu\text{Ci}$ were injected into the fetal (2 animals) or the maternal (3 animals) circulation and samples of fetal and maternal blood and of amniotic fluid were obtained during 2 to 3 hours. In additional experiments labeled platelets were injected into the maternal circulation and samples as mentioned above were obtained after 5 days. No maternofetal or omaternal transfer of ^{51}Cr tagged platelets across the hemochorial placenta could be demonstrated whereas ^{51}Cr Chromium ions appear to cross the placenta. The radiation dose received by a 300 g fetus was estimated to be 11 mrem based on the finding that the sum of total circulating radioactivity in the fetus and radioactivity in amniotic fluid was always less than 1% of the amount of radioactivity injected into the mother. Accepting the analogy between the placentation of rhesus monkey and man it can be estimated that this procedure would result in a radiation dose of approximately 7 mrem for a 1700 g fetus. This would seem to be sufficiently low to allow determination of platelet survival by means of the ^{51}Cr platelet technique in third trimester human pregnancy.

the fetal circulation pertains to a different clinical problem. In preeclampsia a significantly reduced platelet count has been reported (5). It has been suggested that this could be due to an increased consumption of platelets in maternal organs or in the placenta (7). Evidence to verify or deny this hypothesis can be produced by means of determination of platelet life span which involves injection of ^{51}Cr labeled autologous platelets with an activity of approximately $20\ \mu\text{Ci}$ into the maternal circulation (4, 6). Such studies have not been performed in pregnant women because the ethics of human experimentation during pregnancy properly restrict the use of any research procedure, in particular when it involves the use of radioisotopes, until it reasonably can be expected to have no adverse effect on the fetus.

Therefore the present study was designed to obtain experimental data on the permeability or impermeability of the term hemochorial placenta to platelets and to $^{51}\text{Chromium}$ ions in an appropriate experimental animal model. Since the experiments could only be performed with maternal platelets an additional comparative study was made on fetal and maternal platelet size.

METHODS

Experiments were carried out in 7 pregnant rhesus monkeys (*Macaca Mulatta*) obtained from the Primate Center TNO at Rijswijk, The Netherlands. The duration of gestation was known on the basis of timed matings or early rectal palpation and ranged from 145 to 160 days. Acute experiments were performed in 5 animals. 2 animals were subjected to a study of longer duration.

Surgical procedure

The 5 animals subjected to acute experiments were fasted from midnight to surgery the following morning. After premedication with phencyclidine and atropine sulfate surgical anesthesia was induced with 0.2 to 0.5 g fluothane in oxygen following tracheal intubation. Body

In contrast to the long standing evidence that red blood cells can cross the human placenta (8) and recent proof of active fetomaternal transfer of lymphocytes and granulocytes (14) direct evidence of passage of platelets across the hemochorial placenta is not available. An indication that in man fetal platelets might reach the maternal circulation can be derived from the sporadic occurrence of immune neonatal thrombocytopenia, a condition proposed to be similar in pathogenesis to erythroblastosis fetalis except that fetal platelets rather than red cells should provide the antigenic stimulus. The importance of the question as to whether maternal platelets can cross the placenta and reach

warmth was maintained with a heating pad under the animal. The uterus was exposed through a midline abdominal incision and both placentas and interplacental vessels were located by transillumination (11). A chorionic interplacental vein was exposed through a small incision in the myometrium and catheterized with a silicone rubber T tube (inner diameter 0.8 mm, outer diameter 1.3 mm). This technique allows sampling of fetal blood without disturbing flow in the catheterized vessel or destroying the integrity of the amniotic sac. A 16-G polyvinyl catheter advanced into the inferior vena cava from a saphenous vein provided access to the maternal circulation. During the experiments amniotic fluid was obtained by repeat puncture through the lower uterine segment. After the experiments the fetus was either left in the uterus to be vaginally delivered or a Cesarean section was performed. All mothers survived, of the fetuses three survived and two died shortly after the experiments were finished.

Preparation, administration and sampling of ^{51}Cr platelets

Maternal monkey platelets were labeled with ^{51}Cr according to the technique described for human platelets by Aster & Jandi (4) and de Koning (6) which will be only briefly summarized here.

Approximately 100 ml of venous ACD blood were drawn by femoral venipuncture from each animal prior to surgery and a platelet concentrate was prepared by differential centrifugation with excess ACD added before the second centrifugation step. The red cells were retransfused into the mother. The platelet concentrate was incubated with $140 \mu\text{Ci}$ of sodium chromate (^{51}Cr) for 30 min at 22°C , washed with platelet poor plasma and resuspended in fresh platelet poor plasma. Thus ^{51}Cr labeled platelets with an activity of approximately $70 \mu\text{Ci}$ in a volume of 4–5 ml of plasma were obtained. In the acute preparations the ^{51}Cr platelets were injected into the fetal interplacental vein in 2 monkeys and into the maternal inferior vena cava in 3 animals. Following injection of the labeled platelets samples of 5 ml of maternal and of 1 ml of fetal DTA blood and of amniotic fluid were simultaneously obtained at regular predetermined intervals over a 2 to 3 period of time. In 2 animals autologous ^{51}Cr platelets were injected into the maternal circulation under brief meclothidine analgesia and gestation was allowed to continue for 5 days. After that period of time a cesarean section was performed and simultaneous samples of 5 ml of maternal and of 1 ml of fetal blood and of amniotic fluid were obtained.

Radioactivity in all samples was simultaneously assayed in a well type gamma scintillation counter with corrections made for background radiation. The concentration of radioactivity in maternal blood was determined separately in the total cell fraction, in the erythrocytes and in the platelet poor plasma. Since virtually no activity above background level was found in the erythrocytes the amount of platelet bound ^{51}Cr radioactivity could be calculated by correcting the amount of radioactivity in the total cell fraction for the activity in the 70% of the total platelet poor plasma volume which was estimated to remain adherent. A similar procedure was followed for the assay of platelet bound radioactivity in the fetal samples

except that red cell radioactivity could not be separated determined due to the small sample volume. Estimates of maternal blood volume and of amniotic fluid volume were based upon maternal weight, fetoplacental blood volume was estimated from fetal weight. On the basis of these estimates radioactivity found in maternal and fetal blood and in amniotic fluid was calculated as a percentage of injected dose.

Measurement of platelet volumes

Maternal and fetal platelet volumes were estimated in three mother-fetus pairs and compared with values obtained in three non pregnant female monkeys. Sequential counting of platelets in samples of platelet rich plasma prepared from blood collected in 3.8% sodium citrate was performed with a Coulter FN (70 μ tube) coupled to a Channelyser C 1000. Thus curves were obtained expressing the relative frequency of platelet volumes which range from 1.75 to 1.79 μ .

RESULTS

The concentration of radioactivity in blood and plasma is presented as the ratio of radioactivity in each sample to the total amount injected (Table 1). With these data the approximate percentage of injected radioactivity found in each of the various compartments was computed.

Injection of ^{51}Cr platelets into the maternal circulation

After 8 minutes between 70 and approximately 80% of the total amount of radioactivity injected appeared to be present in the circulating blood after 4 hours total circulatory activity was still between 60 and 90%.

In two monkeys 30 and 20% respectively of the total amount of radioactivity administered was still present in maternal blood after 5 days. During the first three hours after injection of the labeled platelets into the maternal vena cava approximately 10% of the total radioactivity in maternal blood was found in the platelet poor plasma fraction and after 5 days radioactivity in plasma was 5 and 7% respectively of the total blood radioactivity at that time. Cell bound radioactivity could not be demonstrated in any of the fetal samples. The concentration of radioactivity in fetal plasma varied between 1 and 10% of the concentration in maternal plasma during the period of two to three hours following the injection of the isotope into the maternal circulation and in monkey no. 2323 where it rose to 72% at 4 hours. Total circulating activity in the fetus was computed to vary between 0.003% of the total

Table 1 Ratio of c p m per ml/c p m in total dose of ^{51}Cr administered $\times 10^{-4}$ after administration of μCi of ^{51}Cr platelets

	Maternal weight (kg)	Fetal weight (g)	Ratio in maternal blood/ Ratio in maternal plasma						Ratio in fetal plasma					
			8	30	60	120	180 min	5 days	8	30	60	120	180 min	5 days
<i>Cr platelets injected into maternal circulation</i>														
1	8.8	385	<u>16.04</u> 0.57	<u>14.75</u> 0.33	—	<u>14.66</u> 0.6	<u>14.34</u> 0.0	—	0.02	0.01	0.02	0.02	0.01	—
	8.5	500	<u>17.97</u> 0.33	<u>14.25</u> 0.24	<u>14.97</u> 0.8	<u>15.58</u> 0.31	—	—	0.01	0.01	0.02	0.01	—	—
3	5.5	400	<u>79.90</u> 0.25	<u>29.30</u> 0.21	<u>29.15</u> 0.34	<u>27.35</u> 0.19	—	—	0.02	0.02	0.03	0.04	—	—
41	7.1	440	—	<u>70.37</u> 0.55	—	—	—	<u>7.09</u> 0.5	—	—	—	—	—	0.13
53	7.4	510	<u>19.56</u> 0.51	<u>19.60</u> 0.21	—	—	—	<u>4.70</u> 0.24	—	—	—	—	—	0.03
			Ratio in maternal plasma						Ratio in fetal blood/Ratio in fetal plasma					
<i>Cr platelets injected into fetal circulation</i>														
2	5.8	340	0.06	0.07	0.06	0.05	0.04	—	370.1	—	—	—	—	—
11	6.7	480	0.08	0.08	0.08	0.09	0.04	—	<u>317.91</u> 16.24	<u>233.54</u> 6.70	<u>244.97</u> 5.29	<u>241.71</u> 5.83	<u>221.50</u> 5.18	—

radioactivity administered to the mother at 8 min and up to 0.03% at 5 days after injection.

Radioactivity could not be detected in amniotic fluid samples taken 8 min after injection. In two animals a small amount of radioactivity was demonstrated in amniotic fluid samples obtained from 30 min to 3 hours after injection; ratios varied from 0.1 to 0.003. One amniotic fluid sample drawn 5 days after injection had a ratio of 0.02 which with amniotic fluid volume of 70 ml represents a total amount of radioactivity in the amniotic fluid of this animal of 0.01% of the total dose administered.

Injection of ^{51}Cr platelets into fetal circulation

In the two experiments performed between 65 and 75% of the total dose of radioactivity administered was recovered in the circulating fetal blood. Between 2 and 5% of the activity in fetal blood was present in the platelet poor plasma fraction. No bound activity was detected in maternal blood. Radioactivity was present in the amniotic fluid 8 min after injection in one animal and after 30 min in two monkeys; the ratios varied between 0.11 and 0.33 over a sampling period of 3 hours.

The curves of distribution of platelet volumes in pregnant and in non-pregnant animals were similar. All fetal curves showed a small shift to the left as compared with the maternal distribution curves (Fig. 1). The average mode of fetal platelet volume was $3.5 \mu^2$ and that of the mother $4.2 \mu^2$.

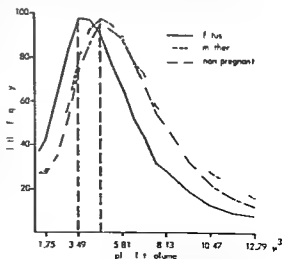


Fig. 1 Distribution of platelet volumes in a rhesus monkey mother-fetus pair and in a nonpregnant animal.

DISCUSSION

Fetomaternal transfer of platelets cannot be experimentally studied in man because the fetal circulation is not accessible unless under the most exceptional circumstances. Also techniques for studying fetomaternal transfer of white cells using the fluorescent Y chromatin as a marker cannot be applied relative to platelets (14). An experimental study on maternofetal transfer in man could technically be done by injecting autologous ^{51}Cr platelets into the maternal circulation a few days before or during delivery and counting a cord blood sample after delivery of the baby. Since no data were available on the possible extent of transplacental passage of ^{51}Cr platelets or on the behavior of free $^{51}\text{chromium}$ this approach was considered unacceptable because of the possible radiation hazard in the fetus. For these reasons the rhesus monkey was chosen as an experimental model. The close similarity between fetal and maternal placental circulation vasculature and villous morphology in this animal and man has been demonstrated (10). Assuming that labeled and nonlabeled platelets are indistinguishable no maternal platelets appear to cross the hemochorial placenta in either direction under the conditions of the present experimental design. It appeared technically impossible to prepare a labeled fetal platelet suspension due to the small fetal blood volume. Though the results of measurement of platelet volumes show that the fetus has a somewhat larger proportion of smaller platelets in comparison with the mother the difference is too small to expect any influence on placental transfer.

To explain the occurrence of isoimmune neonatal thrombocytopenia which has an incidence of approximately 1 or 2 per 10 000 births (12) it could be hypothesized that under sporadic and unknown circumstances fetal platelets are actively transferred to the maternal circulation. However a more likely explanation is that fetal platelets constitute part of small amounts of fetal blood that have been demonstrated to leak through minute breaks of villous vessels (13). Since this appears to occur frequently and since maternal fetal incompatibility of platelet antigens is not unusual (12) antigenicity should be low.

$^{51}\text{Chromium}$ appears to cross the hemomonochorial placenta, whereas no significant amounts are said to be transferred across the hemotrichorial placenta of the rat (3). The radiation dose received

by the monkey fetus when 20 μCi of $^{51}\text{platelets}$ administered to the mother can be estimated on the basis of the finding that no platelet-associated radioactivity passes across the placenta and the sum of the total circulating radioactivity in the fetus and the radioactivity in the amniotic fluid is less than 1% of the amount of radioactivity injected into the mother. The radiation dose to the fetus will be virtually equal to the sum of the average radiation dose received from the radioisotope in maternal tissues (compartment A) and the radiation dose received from free ^{51}Cr ions transferred to the fetal tissues and the amniotic fluid (compartment B).

The biological half life (T_{bi}) of ^{51}Cr in compartment A can be estimated as being 30 days on the basis of known data on ^{51}Cr labeled red cell survival with 50% spleen uptake (9). This is probably an overestimate. The physical half life (T_{ph}) of ^{51}Cr is 27.8 days. Therefore effective half life (T_{eff}) can be calculated to be equal to 15 days. Because the rate with which the fetus eliminates the ^{51}Cr radioactivity is unknown and probably low we shall assume T_{bi} in compartment B to be infinite. It then follows that T_{eff} in compartment B is equal to 28 days.

Compartment A contains approximately 99% of the total radioactivity administered, thus (1) $15 \times 0.99 \times 1.44 = 21.4 \mu\text{Ci days}$ for a dose of 1 μCi . The factor 1.44 represents the integral with which the initial decay rate has to be multiplied to give total decay.

Compartment B contains approximately 1% of the total radioactivity, thus (2) $28 \times 0.01 \times 1.44 = 0.4 \mu\text{Ci days}$.

With these data the dose of radioactivity received in rem per μCi day can be derived by comparison and interpolation from existing tables (1). The fetal dose received from compartment A will be equal to (3) $1.2 \times 10^{-5} \times 21.4 = 0.25 \text{ m rem}/\mu\text{Ci}$.

The radiation dose received by the fetus from compartment B depends on fetal mass and dimensions. The dose for a 300 Gm fetus is $2.2 \times 10^{-5} \text{ rem}/\mu\text{Ci}$ day resulting in (4) $2.2 \times 10^{-5} \times 0.4 = 0.9 \text{ m rem}/\mu\text{Ci}$. The radiation dose for a 1700 g fetus is $2.9 \times 10^{-5} \text{ rem}/\mu\text{Ci}$ day and therefore this fetus will receive (5) $2.9 \times 10^{-5} \times 0.40 = 0.12 \text{ m rem}/\mu\text{Ci}$ from compartment B.

After administration of 20 μCi of ^{51}Cr incorporated platelets to the mother the radiation dose to a 300 Gm rhesus monkey fetus can be computed by combining the dose from compartment A (3) and compartment B (4), thus (6) $20 \times (0.25 + 0.9) = 23 \text{ m rem}$.

If we extrapolate our monkey data to a human fetus with a weight of 1700 g the dose received from compartment A (3) would not change but the contribution from compartment B would be only 0.12 mrem/ μCi (5) and the total dose could be estimated as $70 \times (0.25 + 0.12) = 7.4$ mrem.

For fetuses weighing more than 1700 g the total radiation dose received would be lower because of their greater mass and dimensions (1). In man a fetal weight of 1700 g is normally found at approximately 36 weeks of pregnancy. If we accept the analogy between placentation in man and rhesus monkey the radiation hazard to the human fetus due to administration of $20 \mu\text{Ci}$ of ^{51}Cr labeled platelets to the mother appears to be sufficiently small to allow termination of platelet life span with ^{51}Cr autologous platelets in the third trimester of pregnancy.

ACKNOWLEDGEMENTS

The authors wish to thank Dr L. M. van Putten for his help in estimating the fetal radiation dose and Mrs P. Jansz for her valuable technical assistance.

REFERENCES

- Absorbed dose per unit cumulated activity for selected radionuclides and organs. MIRD Pamphlet 11. Society of Nuclear Medicine Inc. New York 1975.
- Al Rashid R. A. Thrombocytopenic purpura in the newborn. *Paediatrician* 4: 189 1975.
- Assali N. S., Dilts P. V. Jr., Plentl A. A., Kirschbaum T. H. & Gross S. J. Physiology of the placenta. In *Biology of Gestation* (ed. N. S. Assali) vol. 1 p. 761. Academic Press, New York 1968.
- Aster J. A. & Jandl J. H. Platelet sequestration in man. I. Methods. *J Clin Invest* 43: 843 1964.

- 5 Bonnar J., McNicol G. M. & Douglas A. S. Coagulation and fibrinolytic systems in pre-eclampsia and eclampsia. *Br Med J* 2: 12 1971.
- 6 Koning J. de. Diagnostic and therapeutic aspects of idiopathic thrombocytopenia. 41 Thesis Leiden 1975.
- 7 McKay D. G. Physiological intravascular coagulation in normal pregnancy. In *Controversy in Obstetrics and Gynecology II* (ed. E. Reid and C. Christian) p. 285. W. B. Saunders Company Philadelphia, London, Toronto 1974.
- 8 Naeslund J. & Nylin G. Investigations of permeability of placenta with aid of red blood corpuscles tagged with radioactive phosphorus. *Acta Med Scand Suppl* 170: 390 1946.
- 9 Protection of the patient in radionuclide investigations. ICRP Publication 17. Pergamon Press, Oxford, New York 1971.
- 10 Ramsey E. M. & Harris J. W. Comparison of uteroplacental vasculature and circulation in the rhesus monkey and man. *Contrib Embryol* 38: 59 1966.
- 11 Reynolds S. R. M., Paul W. M. & Huggett A. St. G. Physiological study of monkey fetus in utero procedure for blood pressure recording, blood sampling and injection of fetus under normal conditions. *Bull Johns Hopkins Hosp* 95: 256 1954.
- 12 Sitarz A. L., Driscoll J. M. & Wolff J. A. Management of isoimmune neonatal thrombocytopenia. *Am J Obstet Gynecol* 124: 39 1976.
- 13 Woodrow J. C. & Finn R. Transplacental haemorrhage. *Br J Haematol* 12: 297 1966.
- 14 Ziliacius R., de la Chapelle A., Schroder J., Tihkainen A., Kohne E. & Kleihauer H. Transplacental passage of foetal blood cells. *Scand J Haematol* 15: 333 1975.

Submitted for publication July 4 1977

Henk C. S. Wallenburg
Dept of Obstetrics and Gynecology
AZR Dijkzigt
Dr Molewaterplein 40
Rotterdam 3001
The Netherlands

Phadebact® Streptococcus Test



**För gruppering av streptokocker
enligt Lancefield**

**Phadebact® Streptococcus Test,
snabbt co-agglutinationstest av slidetyp
för gruppering av A, B, C och G-
streptokocker enligt Lancefield**

IMPROVED METHOD FOR HYSTEROGRAPHIC EVALUATION OF UTERINE SCAR

Yoram Beyth

From the Department of Obstetrics and Gynecology Hadassah University Hospital Jerusalem Israel

well healed uterine scar is known to be one of the factors for successful vaginal delivery in pre-Cesarean section patients. An uneventful operative course used to be accepted as a guarantee of satisfactory scar repair while symptoms of endometritis were regarded as suggestive of poor healing (1-4). Hystero-uterography performed after Cesarean section was introduced for evaluation of the scar in order to improve prediction of the prognosis of a subsequent vaginal delivery (1-6). Hystero-uterography delineates the configuration of the uterine cavity showing the anterior aspect of the lower uterine segment (1-6). Several irregularities have been described on post-Cesarean hystero-uterographies which are interpreted as due to scarring and as having prognostic significance for vaginal delivery. In this communication an improved diagnostic method

is described which defines more exactly the relationship of uterine cavity irregularities to the scar.

METHOD

At the time of the Cesarean section after closure of the first layer of the uterine incision three Tantalum clips (Hemoclip Edward Weck & Co New York) 5 mm in length are clipped into the myometrium one at each end of the incision and the third at an intermediate site. The second layer of the uterine incision is then closed leaving the clips embedded in the myometrium. Six months after surgery hystero-uterography is performed with anteroposterior (Fig. 1) and lateral views.

DISCUSSION

Hystero-uterography is an accepted method for evaluation of the uterine scar after Cesarean section and

Fig. 1 Anteroposterior view. Hystero-uterogram performed six months after delivery by Cesarean section. Three Tantalum clips are embedded in the myometrium at the site of a lower segment transverse scar: one clip at each end and the third at an intermediate site.

contributes to decision making in the management of a subsequent delivery (1-6). Defects of uterine cavity configuration have been detected in up to 100% of instances (4) but their significance is controversial. The defects have been graded into four classes according to their depth (2-5) and it has been assumed that the depth correlates inversely to the thickness of the uterine wall at the scar.

Hystero-graphy performed after insertion of clips as described above provides additional information: first as a marker the clips permit precise localization of the scar and its relationship to hystero-graphic defects. Second, knowing the length of the clips it may be possible to estimate the minimal thickness of the uterine wall in the region of the defect. The thickness of the myometrium may be more significant prognostically than the depth of the hystero-graphic defect. Finally, the distance between the outermost clips indicates scar length. An incision 10-12 cm long at surgery shrinks to 2-3 cm at hystero-graphy six months post-operatively. A correlation between the outcome of subsequent pregnancies and scar length may provide information which will give hystero-graphy greater predictive value in the future.

REFERENCES

- 1 Baker K. Vaginal delivery after lower uterine section. *Surg Gynecol Obstet* 100: 670 1955
- 2 Camilleri A P & Busuttill T. Twice a Cesarean. *J Obstet Gynaecol Br Comm* 75: 132 1978
- 3 Durkan J P. Hystero-graphy after Cesarean section. *Obstet Gynecol* 24: 836 1964
- 4 Poidevin L O S & Bockner V V. A hystero-graphic study of uteri after Cesarean section. *J Obstet Gynaecol Br Comm* 65: 278 1958
- 5 Surbu P, Vasiliu C & Goranov M. The uterus after Cesarean operations. A radiological study. *Gynecol Obstet Biol Repr* 1: 63 1977
- 6 Zilberman A, Sharf M & Polishuk W Z. Evaluation of Cesarean section scar by hystero-graphy. *Gynecol* 32: 153 1968

Submitted for publication February 15 1977

Y Beyth
Department of Obstetrics and Gynecology
Hadassah University Hospital
P O Box 499
Jerusalem
Israel

PLASMA LEVELS OF NON CONJUGATED OESTRONE IN HIGH RISK PREGNANCIES

O Axelsson B S Lindberg H A Nilsson
and E D H Johansson

From the Department of Obstetrics and Gynaecology University of Uppsala Uppsala Sweden

Abstract Plasma levels of non conjugated oestrone¹ were measured with a radio immunological method in women in complicated pregnancies during the last trimester. Comparison was performed between values of normal pregnancies from a previous report (3) and those of pathological pregnancies. Women with severe pre eclampsia were found to have low values and to some extent the oestrone level could predict fetal outcome. In pregnant women with long standing diabetes mellitus the plasma levels of oestrone seemed to be higher than those of uncomplicated pregnancy. In women with pregnancies complicated by Rh isosensitization oestrone plasma levels were similar to those found in normal pregnancies.

Measurements of urinary oestrogens have long been used as an aid in the care of high risk obstetric patients (9-18). To collect a 24-hour urine sample is inconvenient and it is easy to lose urine voided during defecation. Drugs administered to the mother sometimes interfere with the analyses (1-4). Changes in renal clearance of oestrogens may occur during abnormal pregnancies (5). Because of this plasma oestrogen assays would be more suitable to use in obstetrics as indices of fetal wellbeing. In recent years many investigations of plasma oestrogens and high risk pregnancies have been published. However only few of them have dealt with oestrone (8, 17, 19, 20, 22, 25). We have reported (3) of a rapid and reliable radio-immunoassay for plasma oestrone and its application to normal pregnancy. In the present investigation this method was used to measure plasma levels of non conjugated oestrone in women with high risk pregnancies.

MATERIAL AND METHODS

Subjects

The majority of the patients were treated and delivered at University Hospital of Uppsala. A few patients were delivered in large departments of Obstetrics and Gynaecology at other hospitals. Ante-cubital vein blood was withdrawn into heparinized glass tubes. Plasma was separated

by centrifugation and stored at -20°C until assayed. The plasma specimens were obtained during different weeks of pregnancy. Most patients were sampled at least twice at varying intervals. The diagnosis and classification of pre eclampsia were made according to the recommendations of the U.S. Committee on Maternal Welfare issued in 1952 (10). All mothers with Rh isosensitization had anti Rh antibodies which were demonstrated by the indirect Coombs technique. The infants born alive were Rh positive and had a positive direct Coombs test. The still born infants showed signs of severe haemolytic disease at autopsy.

The White classification was used for the patients with diabetes mellitus (16).

Assay method

A radioimmunoassay was used to estimate oestrone in plasma. The per cent crossreaction of the antiserum was calculated to be 3.3 for oestradiol 17 β and 1.4 for oestrol (3).

Statistical methods

Mean plasma levels of oestrone in the different pathological groups were statistically tested against the means in the corresponding gestational weeks of normal pregnancy (3). In order to test the hypothesis of no difference between groups, analyses of variance were performed. If this hypothesis was rejected 95% simultaneous confidence limits for differences between group means were calculated according to the method of Scheffe (21).

RESULTS

Pre eclampsia

The plasma levels of non conjugated oestrone in patients with mild pre eclampsia are shown in Table 1 and Fig. 1. During the last month of pregnancy the mean values and most of the individual values were spread evenly within the normal range. In patients with severe pre eclampsia (Table 1, Fig. 2) the mean plasma levels of oestrone were generally

The following abbreviations and trivial names are used: oestrone = 3-hydroxy-13,5(10)-oestratriene-17-one; oestradiol 17 β = 13,5(10)-oestratriene-3,17 β -diol; oestrol = 13,5(10)-oestratriene-3,16 α ,17 β -triol; progesterone = 4-pregnene-3,20-dione.

Table 1 Plasma levels in ng/ml of unconjugated oestrone in pregnant women with pre eclampsia
The figures in parenthesis are the corresponding means from normal pregnancy (3) n =number of patients

Weeks of pregnancy	Mild ($n=86$)			Severe ($n=20$)		
	No of samples	Mean	Range	No of samples	Mean	Range
30	2	5.3 (6.8)	2.8-8.6	4	5.0 (6.8)	1.4-7.0
31	1	4.2		2	3.1	2.0-4.2
32	1	4.8 (6.3)		3	3.9 (6.3)	1.4-5.8
33	2	3.3	3.2-3.4	3	6.4	2.8-9.7
34	3	7.5 (6.2)	3.2-12.8	3	3.7 (6.2)	2.0-6.2
35	11	6.0	1.4-11.8	4	4.3	2.2-9.0
36	9	7.6 (6.6)	1.6-14.5	7	5.2 (6.6)	1.2-14.0
37	20	9.4 (7.7)	2.6-28.5	4	8.6 (7.7)	2.2-15.3
38	29	10.4 (9.4)	1.6-34.5	4	6.9 (9.4)	6.0-8.6
39	34	9.6 (8.4)	0.8-20.3	6	8.5 (8.4)	3.8-17.6
40	30	8.9 (8.6)	1.0-23.5	1	21.0 (8.6)	
41	18	12.1 (11.1)	2.6-32.0			
42	4	8.8 (8.5)	5.0-13.0			

lower than those of the normal series. Most individual values were found in the lower part of the normal range. No recordings exceeded the upper normal limit but a few ones fell below the lower limit.

The differences in oestrone plasma concentrations between pre-eclamptic and healthy women were not statistically significant. Two infants of mothers with pre-eclampsia were stillborn. In one of these women the plasma levels of oestrone were at the lower normal limit. Prior to the death of the fetus the values fell below this limit (Fig. 2). In the other case the oestrone plasma concentrations were

within the normal range and the death of the fetus could not be predicted by decreasing oestrone plasma levels. This infant, however, showed multiple malformations which was considered as the cause of death.

The outcome of pregnancy in the severe pre-eclamptic patients with at least one plasma oestrone value of 2.5 ng/ml or less after the 35th week of gestation is compared to the outcome of those with higher values in Table II. This was performed in an attempt to define a "fetal danger zone" and values of 2.5 ng/ml or less were associated with stillbirth, low Apgar scores or low birth weights in 11% of

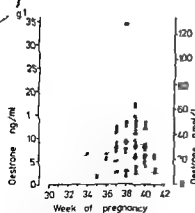


Fig. 1 Plasma levels of unconjugated oestrone in patients with mild pre-eclampsia. In this and the following figures the mean and spread (± 2 S.D.) in healthy pregnant women are indicated as background (3).

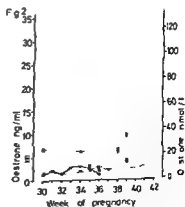


Fig. 2 Plasma levels of unconjugated oestrone in patients with severe pre-eclampsia. The line connects the values for one case of intrauterine death.

Table II The outcome of pregnancy in pre eclamptic patients in relation to the plasma levels of oestrone at the 35th week of pregnancy

Plasma level of oestrone (ng/ml or less at last sample more than ng/ml)	Patient group	Patients	Still born infants	Infants with an Apgar score of 6 or less at 1 min	Birth weight of infants		Uneventful delivery none small for date infants
					Between 1 and 2 S D below normal	More than 2 S D below normal	
Mild		4	0	0	2	1	1
Severe		3	1	1	0	2	1
Mild		76	1 ^a	0 ^a	14	6 ^b	54
Severe		12	0	4	5	5	2

According to Engstrom & Sterky (7)

a) Case of severe malformation

b) Case of a complicated breech presentation

s When all recorded values were above 2.5 ng/ml the corresponding figure was 36%. In the reference group of women with uncomplicated pregnancies less than 5% occasionally showed oestrone plasma values of 2.5 ng/ml or less after the 35th week of pregnancy.

Diabetes mellitus

Plasma levels of oestrone in pregnant women with diabetes mellitus are shown in Table III and Figures 3 and 4. In patients with a short duration of diabetes (White groups II and C) the mean values were fairly constant from the 30th to the 40th week of pregnancy and tended to be higher than those of normal pregnancy. The individual values

were predominantly within the normal limits although a few women had remarkably high values. Two cases of intrauterine fetal death occurred in this group. These patients had oestrone plasma levels in the upper normal range. A moderate increase in plasma oestrone was noticed prior to the fetal death (Fig. 3). In women with longstanding diabetes mellitus (White groups D, F and R) the mean levels were even higher. No rise in the mean plasma concentration of oestrone with advancing pregnancy was observed during the last trimester. The majority of the values were found in the upper normal range. Some patients showed notably high values. The differences in plasma oestrone levels between diabetic and healthy women were not statistically significant.

Table III Plasma levels in ng/ml of unconjugated oestrone in pregnant women with diabetes mellitus. Figures in parenthesis are the corresponding means from normal pregnancy (3). n = number of patients.

Weeks of pregnancy	White groups B and C (n=39)			White groups D, F and R (n=8)		
	No. of samples	Mean	Range	No. of samples	Mean	Range
II	10	3 (6.8)	5.4-16.8	3	16.0 (6.8)	1.7-35.0
3	12	7	2.6-74.1	3	15.3	1.6-31.6
5	7	5 (6.3)	3.8-11.4	4	12.3 (6.3)	6.2-4.5
6	9	8	1.8-16.5	3	13.5	7.4-24.4
9	9	9 (6.7)	3.2-17.8	11	6 (6.2)	4.4-23.5
14	9	1	2.0-32.0	5	17.6	6.8-31.3
22	10	5 (6.6)	1.6-74.0	4	15.9 (6.6)	11.8-78.8
19	9	0 (7.7)	1.4-73.3	4	8.7 (7.7)	11.0-10.8
15	7	0 (9.4)	1.8-17.8	1	18.7 (9.4)	-
9	9	0 (8.4)	3.2-18.5			
3	11	4 (8.6)	3.2-23.3			

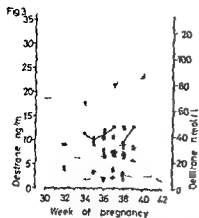


Fig 3 Plasma levels of unconjugated oestrone in patients with diabetes mellitus (White groups II and C). The lines connect values from two cases of intrauterine fetal death.

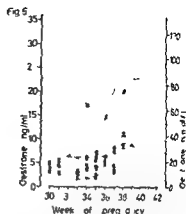


Fig 5 Plasma levels of unconjugated oestrone in patients with mild to moderate Rh immunization (cord Fb > 80 g/l).

Rh immunization

The results of the plasma oestrone estimations in patients with Rh immunization are shown in Table IV and Figs 5 and 6. In mothers of infants with mild to moderate hemolytic disease the mean levels of plasma oestrone were mostly lower than those of the normal series. The differences were not statistically significant. Most values fell within the normal range. The oestrone plasma levels in women with severe Rh immunization differed only slightly from those of the reference group. Three patients were sampled 2-4 days after fetal demise. At that time the oestrone plasma values were still within the normal range and in one case no decrease could be observed (Fig 6).

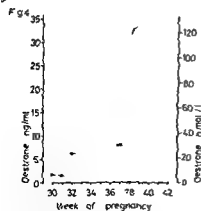


Fig 4 Plasma levels of unconjugated oestrone in patients with diabetes mellitus (White groups D, F and R).

DISCUSSION

Previous investigations on plasma or blood oestrone in women with pre-eclampsia have revealed that low or subnormal values are common, especially when pre-eclampsia is associated with stillbirth, neonatal asphyxia or low birth weight (8, 19). Kubli & Keller (17) presented similar results. In their report, however, oestrone and oestradiol 17 β were not assayed separately. In the above mentioned reports, total (unconjugated + conjugated) oestrone in plasma or blood were measured. The low oestrone levels are supposed to reflect an impaired placental function. Although the present study gives plasma levels of unconjugated oestrone, the results are in good agreement with previous works in that low or subnormal values are common in patients with severe pre-eclampsia.

An attempt to establish a fetal death pattern showed that intrauterine death, neonatal asphyxia or low birth weight frequently occurred after levels of 2.5 ng/ml or less after the 35th week of pregnancy. One case of fetal death occurred with a higher oestrone plasma level, thus far, but the fetus had severe malformations. Signs of fetal distress, low Apgar scores or low birth weight were observed in about 35% of the patients even though all recorded oestrone plasma values exceeded 2.5 ng/ml. Lonnau et al (14) suggested that measurement of any plasma oestrogen could be used to assess fetoplacental function. Other authors have claimed that measurement of oestrone gives the adequate information about the fetal state (13). Dumont et al (16) have stated that est-

Table IV Plasma levels in ng/ml of unconjugated oestrone in pregnant women with Rh isoimmunization figures in parenthesis are the corresponding means from normal pregnancy (3) n = number of patients

Weeks of pregnancy	Cord blood haemoglobin more than 80 g/l ($n \approx 6^2$)			Cord blood haemoglobin ≤ 80 g/l or less ($n=15$)		
	No of samples	Mean	Range	No of samples	Mean	Range
6		4.3 (6.8)	2.8-6.8	4	6.4 (6.8)	5.0-7.2
9		5.0	2.6-9.0	4	5.2	2.4-7.0
9		4.0 (6.3)	1.4-7.4	8	6.3 (6.3)	1.6-11.2
13		5.0	1.6-13.5	6	7.3	1.6-12.0
14		8.9 (6.2)	1.8-25.5	8	7.2 (6.2)	1.4-13.0
17		5.4	1.8-10.8	4	9.5	3.2-16.3
18		8.6 (6.6)	3.6-21.8	4	9.6 (6.6)	4.6-16.0
17		7.2 (7.7)	3.0-15.3			
16		11.4 (9.4)	3.4-29.8	1	3.0 (9.4)	
5		6.4 (8.4)	3.0-10.0			
2		6.4 (8.6)	4.8-8.0			

unconjugated oestrone in plasma is less suitable for use in this connection because of the large spread of the normal values. Mathur et al (17) have drawn attention to the fact that plasma from pre-eclamptic patients can convert oestradiol 17β to oestrone.

They suggest that the use of oestradiol in the management of high risk pregnancies should be combined with measurement of oestrone. The present study shows that plasma values of unconjugated oestrone may be used as an index of fetal well being in patients with pre-eclampsia although false positive as well as false negative indications are rather common.

Divergent results have been published about plasma levels and urinary excretion of oestrogens in diabetic pregnancy. In a report from 1970 Lyngbye (15) presented a review on the subject. Roy & Kerr (20) found low blood levels of total oestrone in pregnant women with diabetes mellitus. Most values, however, were within the normal range. The authors suggested that the observed low levels seemed to be more related to associated conditions such as pre-eclampsia rather than primarily to the diabetes. In the present study a few diabetic women had their pregnancy complicated with pre-eclampsia. With one exception the oestrone plasma levels in these patients were low or subnormal. In one woman high values were recorded. This patient, however, was also suffering from a renal disease. Wodrig & Goretzlehner (25) presented significantly lowered values of unconjugated oestrone in blood in patients with diabetes mellitus. Our results on the other hand are in agreement with those of Svendsen & Sorensen (22) who found high plasma levels of unconjugated oestrone in two pregnant women with diabetes. They suggest that these high oestrone values might reflect the large placentas found in diabetic patients.

The conflicting findings on oestrone levels in plasma of diabetic women during pregnancy may be due to the fact that diabetes is a heterogeneous disease in regard to duration, severity and principles of treatment. Estimation of the plasma levels of unconjugated oestrone seems to be of no use in predicting the outcome of diabetic pregnancies.

Previous investigations on plasma oestrone in

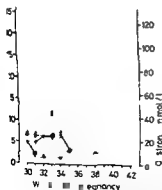


Fig. 6 Plasma levels of unconjugated oestrone in patients with severe Rh immunization (cord Hb ≥ 80 g/l or less) and in patients with intrauterine fetal death due to severe hemolytic disease (E). The values denoted by * are from samples taken 7-14 days after the death of the fetus.

women with Rh immunization are few Fischer Rasmussen (8) who measured total oestrone in plasma found values well within the normal range in two women with Rh immunization. One of these women delivered a healthy infant. In the other case an intrauterine death occurred. These findings are in accordance with the results of the present report which indicate that no correlation exists between the haemolytic process in the fetus and the plasma levels of oestrone in the pregnant woman. Tulchinsky & Korenman (24) observed high and rising plasma values of oestradiol 17β in two cases of intrauterine fetal death. A similar trend was not observed in the three cases of intrauterine fetal death in the present study. Oestrone plasma values recorded after fetal death were within the normal range (Fig. 6). The same observation has been described for oestradiol 17β (24). Persisting normal values of these hormones despite clear evidence of fetal death seem to be connected with the hypertrophied placenta found in these patients. There are no indications that measurement of plasma oestrone itself could be of any use in evaluating fetal prognosis in patients with Rh immunization. As pointed out by Lindberg et al (13) and Tulchinsky et al (23) measurement of the oestradiol 17β /oestrol ratio in plasma possibly in combination with the progesterone/oestrol ratio is helpful for predicting fetal well being in Rh haemolytic disease.

CONCLUSION

The levels of nonconjugated oestrone in maternal plasma will to some extent reflect the fetal state in cases with pre-eclampsia. In pregnant women with diabetes mellitus or Rh immunization the oestrone plasma levels could not be used to predict fetal outcome.

ACKNOWLEDGEMENT

This study was supported by the Ford Foundation, the Swedish Medical Research Council (Grant No. 3495), the Expressen Fund for Prenatal Research and the Allmänna Barnbördshusets Mödravårdsdelegation.

REFERENCES

- Acosta A A, Madeira L B, Besch P K & Buttram V C Jr. Additional observations on hydrochlorothiazide interference with measurement of total urinary placental oestrogens. *Clin Chem* 19: 261 1973.
- Adlercreutz H, Martin F, Tikkanen M & Järkinen M. Effect of ampicillin administration on excretion of twelve oestrogens in pregnancy. *Acta Endocrinol (Kbh)* 80: 551 1975.
- Axelsson O, Lindberg B S, Nilsson B & Johansson E D B. A radioimmunoassay method for oestrone. Plasma levels of nonconjugated oestrone during uncomplicated pregnancy. *Acta Obstet Gynecol Scand* 56: 49 1977.
- Carlström K & Lunell N O. Interference of septic containing hexamethylene tetramine (A) with the estimation of urinary oestrol during pregnancy. *Acta Obstet Gynecol Scand* 56: 187 1975.
- Carrington E R, Oesterling M J & Adams F I. Renal clearance of oestrol in complicated pregnancy. *Am J Obstet Gynecol* 106: 1131 1970.
- Dumont M, Cohen M, Cohen H & Bercu L. Le diagnostic de la souffrance fœtale à partir des dosages plasmatiques des oestrogènes conjugués. *Rev Franc Gynéc* 70: 171 1975.
- Engström L & Sterky G. Standardkurvor för och längd hos nyfödda barn. *Läkartidningen* 61: 1966.
- Fischer Rasmussen W. Gas liquid chromatographic measurement of oestrol, oestrone and oestradiol in the plasma of pregnant women. *Dan Med Bull Suppl* 11: 1972.
- Furuhjelm M. The excretion of oestrol and oestradiol in toxemia of pregnancy and in preeclampsia. *Acta Obstet Gynecol Scand* 41: 370 1962.
- Greenhill J P. *Obstetrics* 13th ed. p 91 & 92. Philadelphia 1965.
- Klopper A, Jandial V & Wilson O. Plasma assay in the assessment of foetoplacental function. *Steroid Biochem* 6: 651 1973.
- Kubli F & Keller M. Plasmooestrogene bei pathologischen Schwangerschaft und unter Geburt. *Arch Gynäkol* 189: 139 1963.
- Lindberg B S, Johansson E D B & Nilsson B. A. Plasma levels of non conjugated oestrone and oestrol in high risk pregnancies. *Acta Obstet Gynecol Scand Suppl* 37: 37 1974.
- Lonaux D L, Ruder H J, Knab D R & Le M B. Estrone sulphate, estrone, estradiol and oestrone plasma levels in human pregnancy. *J Clin Endocrinol Metabol* 35: 887 1972.
- Lyngbye J. Oestrogen metabolism in diabetic pregnancy. *Dan Med Bull* 17: 173 1970.
- Marble A, White P, Bradley R F & Knipf P. Joslin's Diabetes Mellitus p 588. Lea & Febiger Philadelphia 1971.
- Mathur R S, Leaming A B & Williamson B. An assessment of the total estrone, estradiol and oestrol in high risk pregnancy plasma. *J Steroid Biochem* 6: 1421 1975.
- Ostergård B R. Estrol in pregnancy. *Obstet Gynecol Survey* 28: 215 1973.
- Roy E J, Harkness R A & Kerr M G. Concentration of oestrogens in blood and urine of women suffering from pre eclampsia. *J Obstet Gynaecol Comm* 70: 597 1963.

Roy E J & Kerr M □ The concentration of oestrogens in the peripheral blood of the pregnant diabetic woman J Obstet Gynecol Br Comm 71 106 1964

Scheffe H The Analysis of Variance p 66 John Wiley & Sons New York 1963

Svendsen H & Sorensen B The concentration of unconjugated oestrone and 17 β -oestradiol in plasma during pregnancy Acta Endocrinol (Kbh) 47 237 1964

Tulchinsky H Hobel C J Yeager E & Marshall J R Plasma estradiol, estrone and progesterone in human pregnancy II Clinical applications in Rh isoimmunization disease Am J Obstet Gynecol 113 766 1977

24 Tulchinsky D & Korenman S G The plasma estradiol as an index of fetoplacental function J Clin Invest 50 1490 1971

25 Wodrig W & Goretzlehner G Untersuchungen über den Östrogenspiegel im Blutserum und Fruchtwasser bei Diabetes mellitus und Schwangerschaft Z Geburtsh Gynakol 162 ■ 1964

Submitted for publication Sept 9 1976

O Axelsson
Department of Obstetrics and Gynecology
University Hospital
750 14 Uppsala
Sweden

PLASMA STEROIDS IN THE FOETAL AND MATERNAL CIRCULATION AT NORMAL DELIVERY AND ELECTIVE CAESAREAN SECTION

P Coats E Florensa E Youssefnejadian and I Craft

From the Institute of Obstetrics and Gynaecology Queen Charlotte's Hospital London England

Abstract Maternal and foetal plasma collected at normal delivery and at elective caesarean section was assayed for steroid levels. Foetal cord plasma concentrations of progesterone, 17-hydroxyprogesterone, oestrone and oestradiol were higher than maternal values at normal delivery, whereas oestradiol levels were lower. Oestrone concentrations were higher than oestradiol in the foetal circulation at normal delivery and oestrone values were higher than oestradiol at elective caesarean section.

As term approaches and labour progresses a change in the endocrine environment occurs within the foeto-placental unit. The nature of this change has not been variously investigated in the human situation and no agreement has been reached (8, 9, 10). Animal studies indicate a striking rise in 17 β -oestradiol during a contrary action on the myometrium to progesterone (?) and in premature human labour elevated levels of oestradiol have been reported (11). The purpose of this study reported here was to compare oestrone, oestradiol, oestrinol, progesterone and 17-hydroxyprogesterone concentrations in the foetal and maternal circulations at normal delivery and at elective caesarean section to evaluate their interrelationships and their association with parturition.

MATERIALS AND METHODS

5 ml of heparinized blood was collected in each infant and the plasma separated and stored at -20°C prior to analysis. Radioimmunoassay of oestrone, oestradiol, oestrinol, progesterone and 17-hydroxyprogesterone was performed using radioimmunoassay techniques previously reported (12, 13, 14, 15).

RESULTS

The mean and standard deviation of individual steroid hormones in the maternal and foetal plasma

following normal delivery and expressed in ng/ml are shown in Figs 1 and 2. The concentrations of oestrone, oestrinol, progesterone and 17-hydroxyprogesterone were significantly greater in umbilical venous plasma compared with maternal peripheral venous blood, whereas oestradiol levels were significantly lower (Table I). Oestrone concentrations were significantly greater in the umbilical vein compared to the umbilical artery (37.9 ± 9.8 compared with 28.5 ± 10.6 , $t=5.52$, $p<0.01$) but differences in the concentrations of the other steroids in the foetal umbilical vessels were not significant (Fig. 2).

The results from blood taken at elective caesarean section in three subjects, corrected to the nearest whole number, are shown in Table II. Progesterone and oestrinol levels were markedly higher in the foetal than in the maternal circulation. Oestrone levels were also raised in the foetal samples but the range detected (24.6-29.8) was considerably lower than the mean value found following normal delivery (37.9). Oestradiol levels were lower in the foetal than in the maternal circulation at normal delivery.

DISCUSSION

Changes in oestrogen metabolism occur in the foeto-placental unit in late human pregnancy. The finding of higher oestrone concentrations in the foetal circulation following normal delivery than at caesarean section confirms the results of Patten et al. (8). Oestrone is in higher concentration in the foetal than in the maternal circulation and is higher in the umbilical vein compared to the umbilical artery, supporting the concept that changes in oestrone production occur in the placenta in association with spontaneous labour, since there were no such differences at elective caesarean section. The biologically more potent oestradiol was in lower

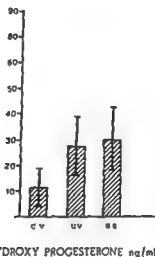
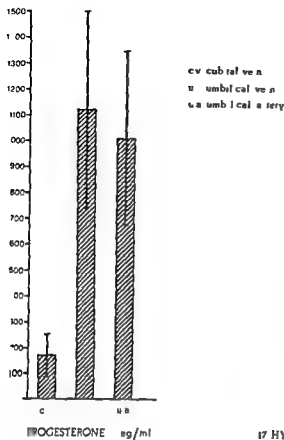


Fig 1 Plasma steroids in the foetal and maternal circulation at normal delivery—progesterone and 17 hydroxy progesterone

concentration in the umbilical vein compared to the artery and higher in the maternal venous plasma at normal delivery which may be evidence for a mechanism metabolising oestradiol in the placenta which protects the foetus

Prostaglandin concentrations are also increased in the foetal circulation in late human pregnancy (4) but the significance of the finding and its possible interrelationship with the changing steroid environment at the onset of spontaneous labour is uncertain. Progesterone and 17 α hydroxyprogesterone are found in higher concentrations in the foetal compared to the maternal circulation (6)

both before and following the onset of parturition and the finding that foetal progesterone values are higher rather than lower following normal delivery than in late pregnancy suggests that this steroid hormone is not so involved in the initiation of parturition in the human as in other animal species.

Oestrogen production in the foeto-placental unit is regulated by foetal adrenal function (1) and maternal arterial and venous levels of ACTH are greater in spontaneous than in induced labour, probably indicating a pituitary-adrenal-foetal metabolism link. It may be that the development of progressive uterine contractions indicative of

Table I Steroid concentrations in maternal cubital vein and umbilical vein plasma at normal delivery (n=19)

Mean \pm S.E.

	Progesterone	17 hydroxy progesterone	Oestrone	Oestradiol	Oestrol
Cubital vein	163 \pm 81	11.1 \pm 7.1	10.9 \pm 5.3	29.8 \pm 15.3	70.6 \pm 6
Umbilical vein	1119 \pm 379	27.6 \pm 10.0	37.9 \pm 9.8	20.1 \pm 7.1	180.0 \pm 47.9
Significance p	<0.001	<0.001	<0.001	<0.02	<0.001

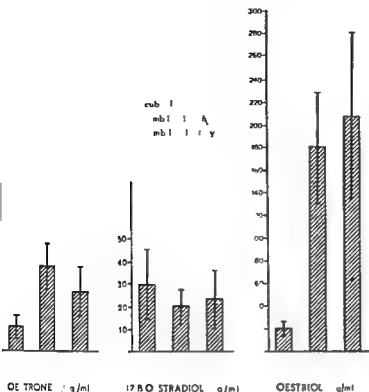


Fig 2 Plasma steroids in the foetal and maternal circulation at normal delivery—oestrogens

our process follow complex sex steroid changes a mechanism similar to that occurring in the 'ep (7) whereby oestrogen dominance could be involved in prostaglandin synthesis (3) thereby increasing the sensitivity of the myometrium to oxytocin (14)

Although the precise mechanisms preceding the conversion of non progressive to progressive uterine activity remain uncertain there is evidence of dynamic changes in oestrogen metabolism occurring in association with parturition but it is probable that others are so transient or localised that they remain undetected in peripheral sampling

ACKNOWLEDGEMENTS

We are grateful to the volunteers who participated in this study and to Upjohn Limited for their support

REFERENCES

1. Arai K, Kuwabara Y & Okinaga S. The effect of adrenocorticotrophic hormones and dexamethazone administered to the foetus in utero upon maternal and foetal oestrogens. *Am J Obstet Gynecol* 113 316 1972
2. Challis J R G. Sharp increase in free circulating oestrogens immediately before parturition in sheep. *Nature* 229 708 1971 (letter)

Table II Concentrations of sex steroid hormones (ng/ml) in maternal and foetal circulations at elective caesarean section (n=3)

mean and range

	Progesterone	17 hydroxy progesterone	Oestrone	Oestradiol	Oestriol
Maternal vein	150 (177-186)	28 (7-66)	15 (10-22)	26 (9-48)	25 (11-38)
Foetal vein	440 (370-520)	3 (19-45)	27 (25-30)	13 (6-19)	209 (172-237)
Foetal artery	377 (311-470)	23 (19-77)	26 (19-31)	16 (10-23)	243 (170-402)
Maternal artery	215 (180-260)	363 (11-84)	14 (17-16)	76 (17-39)	47 (13-104)
Foetal vein	393 (108-880)	46 (9-96)	11 (10-21)	47 (2-77)	11 (40-106)

- 3 Challis J R Harrison F A Heap R B Horton E W & Poyster N L A possible role of oestrogens in the stimulation of prostaglandin F_{2α} output at the time of parturition in a sheep *J Reprod Fert* 30 485 1972
- 4 Craft I L Scrivener R & Dewhurst C J Prostaglandin F_{2α} levels in the maternal and foetal circulations in late pregnancy *J Obstet Gynaecol Br Comm* 80 616 1973
- 5 Emmet Y Collins W P & Sommerville I F Radioimmunoassay of oestrone and oestradiol in human plasma *Acta Endocrinol* 69 567 1972
- 6 Harbert G M McGaughey H S Scoggin W A & Thorton W N Concentration of progesterone in newborn and maternal circulations at delivery *Obstet Gynecol* 23 413 1964
- 7 Keirse M J N C & Turnbull A C Prostaglandins in amniotic fluid during late pregnancy and labour *J Obstet Gynaecol Br Comm* 80 970 1973
- 8 Patten P T Anderson A B M & Turnbull A C Human fetal and maternal oestrogens and the onset of labour *J Obstet Gynaecol Br Comm* 80 952 1973
- 9 Shaaban M M & Klopper A Changes in unconjugated oestrogens and progesterone concentration in plasma at the approach of labour *J Obstet Gynaecol Br Comm* 80 210 1973
- 10 Shearman R P Joons N D & Smith I D Maternal and foetal venous plasma steroids in relation to parturition *J Obstet Gynaecol Br Comm* 79 1972
- 11 Smith I D & Shearman R P Foetal plasma steroid levels in relation to parturition I The effect of gestation upon umbilical plasma Corticosteroid levels following vaginal delivery *J Obstet Gynaecol Br Comm* 81 11 1974
- 12 Tambyraja R A Anderson A B M & Turnbull A C Endocrine changes in premature labour I 4 67 1974
- 13 Youssefnejadian E Florensa E Collins W P & Sommerville I F Radioimmunoassay of plasma progesterone *J Steroid Biochem* 3 893 1972
- 14 Youssefnejadian E Florensa E Collins W P & Sommerville I F Radioimmunoassay of 17 hydroxyprogesterone *Steroids* 20 773 1972
- 15 Youssefnejadian E & Sommerville I F Radioimmunoassay of plasma oestrinol *J Steroid Biochem* 20 659 1973

Submitted for publication March 2 1977

Ian Craft
Academic Department of Obstetrics and Gynaecology
Royal Free Hospital
London NW3
England

ELECTRICAL POTENTIAL DIFFERENCE ACROSS THE MID TERM HUMAN PLACENTA

J Štulc J Švihovec J Drabková J Strábrný J Kobálková I Vido
and A Dolcžal

*From the Department of Pharmacology Faculty of Pediatrics and Department of Anaesthesiology
and Resuscitation and 1st and 2nd Clinic of Obstetrics and Gynecology Faculty of
General Medicine Charles University Prague Czechoslovakia*

Abstract Electrical potential difference across the mid
term human placenta was recorded during hysterotomy
average value registered was 2.7 mV (S.E. of
mean = 0.4 mV $n=7$) fetus negative. From this value and
concentrations of Na K Ca Cl and inorganic
phosphate in maternal and fetal plasma the possible
mechanisms of net transport from mother to fetus are
discussed. It is concluded that of the above ions only the
transport of Na is compatible with simple diffusion.

Material exchanged between mother and fetus
through the placenta must cross the placental barrier. The
mechanism across the barrier may be effected by
various mechanisms (9).

The growing fetus accumulates inorganic ions.
This implies that there is a net flux of ions across
the placental barrier from the maternal to the fetal
side.

Of the ions taken up by the fetus Na K Ca Cl
and inorganic phosphate are accumulated at by far
the highest rates (4). Therefore only these ions will
be further considered.

The mechanisms by which the net flux of ions
across the placenta takes place are not known, but
certain inferences in this respect could be made
from the values of the electrochemical potential
difference across the placenta.

If the most simple mechanism of ion transport
across the placenta would be simple diffusion down
the electrochemical potential gradient. The con-
centrations of K Ca and inorganic phosphate in
fetal plasma however are significantly above those
in maternal plasma, the concentrations of Na and Cl
on two sides of the placenta are approximately
the same (7). From this it follows that the net flux
of these ions across the placenta cannot proceed by

simple diffusion unless there is an electrical poten-
tial difference across the barrier providing the
necessary driving force.

An electrical potential difference across the
placenta (p.d.) exists in many animal species (5, 7, 9).
In humans at term no p.d. significantly different
from 0 mV was detected (6). However the record-
ings of the p.d. across the human term placenta may
not pertain to earlier stages of pregnancy. The tis-
sue separating maternal and fetal blood in the hu-
man placenta becomes highly attenuated with aging
of the placenta (1, 3) and the electrical resistance of
the barrier is likely to decrease at the same time. It
is therefore possible that a p.d. across the human
placenta exists in earlier stages of fetal development
but that it disappears with advancing pregnancy.
For this reason we have measured the p.d. between
maternal and fetal blood at mid pregnancy.

METHODS

The recordings were performed in 7 women undergoing
legal abortion by hysterotomy. The reason for abortion
was rubella in 6 women and schizophrenia in one woman.
The ages of the conceptuses estimated from the date of the
last menstruation ranged from 15 to 22 weeks, the average
fetal weight was 790 g (S.E.M. = 37 g).

The p.d. was recorded with a Tesla BM 483 microvolt
meter connected to the maternal and fetal blood through a
pair of calomel electrodes and a pair of agar bridges
(Portex 00 nylon tubing filled with agar saturated KCl
solution). The agar bridges were inserted into the maternal
cubital vein and into the umbilical artery of the fetus
through guiding hypodermic needles. The bridges were
sterilized before use. The fetus remained in the uterus
during the recording. The values obtained were corrected
for the asymmetry of the electrodes.

RESULTS

In all instances the fetal blood was negative with respect to the maternal blood. The values of the p_d obtained ranged from 0.8 mV to 3.7 mV with an average value of 2.7 mV (S.E.M. = 0.4 mV). There was no correlation between the p_d and the length of pregnancy.

DISCUSSION

The present recordings demonstrate that a p_d does exist in humans. However, except for Na, the p_d observed cannot account for the net flux of the ions from mother to fetus. The fetal side of the placenta being electronegative, Cl and inorganic phosphate move up the electrical potential gradient. In the case of K and ultrafiltrable Ca the p_d would have to be 7.2 mV and 3.7 mV (fetus negative) respectively for the ions to be in equilibrium across the placenta. (The equilibrium potentials were calculated from the concentrations of K (8) and ultrafiltrable Ca (2) in maternal and fetal plasma using the Nernst equation.) Since the p_d observed is below these values, the force of the electrical potential gradient driving K and Ca into the fetus is lower than the oppositely directed force represented by the concentration gradient.

Of the ions considered only the net flux of Na across the placenta is compatible with simple diffusion. For the other ions a non diffusional presumably active mechanism of transport across the placenta has to be assumed.

REFERENCES

- 1 Boyd J D & Hamilton W J. *The Human Fetus*. Hefter Cambridge 1970.
- 2 Delivoria Papadopoulos M, Battaglia F C, B. C. D. & Meschia G. Total protein bound and ultrafiltrable calcium in maternal and fetal plasma. *Am J Physiol* 213: 363 1967.
- 3 Dempsey E W. Ultrastructure of the placenta: consequences for drug distribution. In *Fetal Pharmacology* (ed L. Boréus). Raven Press, New York 1973.
- 4 McCance R A & Widdowson E M. Metabolism of the foetus and newborn. *Med Bull* 17: 133 1961.
- 5 Mellor D J. Potential differences between mother and foetus at different gestational ages in the rat. *Int J Guinea pig J Physiol* 204: 395 1969.
- 6 Mellor D J, Cockburn F, Lees M M & Blyth J. Distribution of ions and electrical potential difference between mother and foetus in the human at term. *Obstet Gynaec Br Comm* 76: 993 1969.
- 7 Meschia G, Wolkoff A S & Barron D H. Difference in electric potential across the placenta of grasshopper. *Nat Acad Sci USA* 44: 483 1958.
- 8 Thalmé B. Electrolyte and acid-base balance in mother and maternal blood. An experimental and clinical study. *Acta Obstet Gynec Scand* 52 (Suppl 1) 1973.
- 9 Widdowson E M. Transport mechanisms in the foetus. *Med Bull* 17: 107 1961.

Submitted for publication March 16 1977

J Štulc
Dept of Pharmacology
Faculty of Pediatrics
Albertov 4
128 00 Prague 2
Czechoslovakia

INTRAUTERINE DEATH DUE TO INFECTION WITH GROUP B STREPTOCOCCI

G Bergqvist G Holmberg T Rydner and V Vaciavinkova

from the Departments of Pediatrics St Goran Hospital and Obstetrics and Gynaecology Sabbatsberg Hospital
Karolinska Institutet and the Department of Obstetrics and Gynaecology
St Erik Hospital Stockholm Sweden

Abstract During the 6-year period 1970-1975 5 cases of intrauterine death caused by group B streptococcal infection were seen in two obstetrical departments in the Stockholm area. During the same period 17638 infants were born in the two departments and in 117 cases intrauterine death occurred. Hematogenous spread of the infection from the mother was the most likely cause in the cases. This figure should be compared with a carrier rate of 1-70% in pregnant women in the Stockholm area.

During the last decade there have been several reports on neonatal group B streptococcal infections from the United States and from various countries in Europe (2, 3, 6, 7, 8). The spectrum of infections varies considerably from simple infections such as low grade omphalitis to severe conditions with a high mortality (3, 9). The children suffering the so called 'early type' have usually contracted the infection in connection with delivery and the same bacteria could then be isolated from the genital organs of the mother (3, 6, 7). However, this bacterium seems to be very common in the urogenital organs both in pregnant and nonpregnant women (1, 4, 5). But the possibility of group B streptococci causing intrauterine infection and abortion is less well documented although Hood et al. (10) referred to it in 1961 in one of the earliest papers on group B streptococcal infection. We have observed some cases in Stockholm and we thought it of interest to report.

MATERIAL AND METHODS

During the six year period 1970-31 1975 altogether 17638 infants were born at two maternity clinics in Stockholm. Among them were 117 cases of late intrauterine death, all of which have been fully reviewed. An autopsy was performed in all cases by a pathologist and bacteriological specimens were taken regularly from the infants for instance from cerebral tissue, cardiac and placental tissue etc.

RESULTS

In altogether 24 cases bacterial infection seems to have contributed to the fetal death. The bacteriological composition of these cases is shown in Table I. No case of *Listeria* was found.

Regarding the group B streptococcal infection three infants were small for their gestational age. None of them were born before term. The five group B streptococcal infants probably died one to four days before delivery. The time between membrane rupture and delivery was less than four hours in all the five streptococcal cases and in two cases only 30 and 45 min respectively.

DISCUSSION

Group B streptococci as well as enterococci were common. The same finding has been made by Hood et al. (8) who isolated group B streptococci from the brains of 11 out of 113 dead fetuses or stillborn infants. The spread to infants with massive infection was probably hematogenous as the mothers had no signs of ascending infection. However, Overall (10) has pointed out the probability of group B streptococci penetrating intact membranes.

Baker et al. (1) calculated the risk of an infant of a group B streptococci carrier mother contracting an infection to be around 1%. They considered the risk of intrauterine death to be small, putting the overall risk of intrauterine death due to infection at

Table I

Streptococci group B	5
Streptococci group D (enterococci)	10
<i>E. coli</i>	5
Other Gram neg. rods	4
Total	24

only 0.13% and the risk of intrauterine streptococcal group II death at only 0.028%. This should be compared with a carrier rate of 15–20% in pregnant women in the Stockholm area (4, 5), a figure 500–700 times higher. Thus the occurrence of sporadic cases of intrauterine death due to group B streptococcal infection hardly warrants general screening programs for group B streptococcal carriers among pregnant women. However in individual cases with previous group B streptococcal infection (intrauterine or neonatal) we keep the mother under observation during pregnancy to detect if she is a carrier.

REFERENCES

- 1 Baker C J, Barrett F F & Yow M D The influence of advancing gestation group II streptococcal colonization in pregnant women. *Am J Obstet Gynecol* 122: 820, 1975.
- 2 Baker C J, Barrett F F, Gordon R C & Yow M D Suppurative meningitis due to streptococci of Lancefield group II: a study of 13 infants. *J Pediatr* 82: 724, 1973.
- 3 Bergqvist G, Hurvell B, Malmborg A S, Rylan

- der M & Tunell R Neonatal infections caused by group II streptococci. *Scand J Infect Dis* 10: 197.
- 4 Bergqvist G, Hurvell B, Thal C & Vahlne V Neonatal infections caused by group B streptococci. *Scand J Infect Dis* 10: 197.
- 5 Bergqvist G, Bollgren I, Hurvell B & Vahlne V Unpublished results.
- 6 Cayeux D Infections neonatales à streptocoques group B: constations étiologiques. A propos d'observations. *Arch Fr Pédiatr* 29: 391, 1972.
- 7 Franciosi E A, Knostman J D & Zimmerman A Group II streptococcal neonatal and infantile infections. *J Pediatr* 87: 707, 1973.
- 8 Hood M, Janney A & Dameron E Hemolytic streptococcus group B associated problems in the perinatal period. *Am J Obstet Gynecol* 82: 809, 1961.
- 9 Howard J B & McCrachen G H Jr Therapy of group B streptococcal infections in infancy. *Dis Child* 128: 815, 1974.
- 10 Overall J C Neonatal bacterial meningitis. *HL* 76: 499, 1970.

Submitted for publication April 4, 1977

V. Vaclavinkova
Dept. of Obstetrics and Gynecology
Sabbatsberg Hospital
Stockholm
Sweden

MATERNAL MORTALITY IN SWEDEN 1955-1974

Stefan Fianu

*From the Department of Obstetrics and Gynecology, Sabbatsberg Hospital,
Karolinska Institutet, Stockholm, Sweden*

Abstract A survey of the causes of maternal death in Sweden during the years 1955-74 is presented. 296 cases where autopsy has been performed in 94% have been reviewed. 219 have been classified as obstetrical deaths, 177 as non-obstetrical. During the period an almost 50% lowering of the maternal death rate occurred. The explanation is a probable improvement in the quality of medical care given in the hospitals and the antenatal clinics.

In the past childbirth was a time of great anxiety and danger. The earliest London records tell us that in the seventeenth century one woman in 40 lost her child at the time of delivery. A century ago the risk was one fifth and the maternal mortality rate was expressed as being 5 per 1000 registered live or stillbirths.

The maternal mortality rate in Sweden according to early Göteborg records was 4% between 1856-65 (at the Carolus Dux Maternity in Göteborg). By 1876 the risk at delivery had fallen to 0.61%. This figure remained almost unchanged for 60 years. It was not until 1936 that a steady and dramatic decline started.

MATERIAL

The material studied consisted of 296 cases from the years 1955-74. It was divided into 10-year periods and correlated with the official number of deliveries published by the National Board of Health and Welfare (Table I).

The maternal mortality rate is expressed as a proportion based on the number of deliveries and the number of maternal deaths within a month of delivery of a liveborn infant or a stillborn foetus at least 35 cm in length. Maternal deaths following abortion were not included. The study was based on a direct analysis of the hospital records of all maternal deaths in Sweden during the 20-

year period 1955-74. The figures for the number of deliveries have been obtained from the official statistics of the National Board of Health and Welfare.

The hospital records were obtained by kind cooperation of the heads of the respective departments.

Autopsy was performed in 94% of the cases with classification according to the complication considered to be the principal cause of death.

RESULTS

During the two 10-year periods 2 222 909 women gave birth with 296 maternal deaths (Fig. 1). This figure shows that the mortality rate which started to fall in 1935 has continued its downward trend up to the present day. After dividing the causes of maternal death into two groups—obstetrical and non-obstetrical—there were 219 obstetrical and 77 non-obstetrical causes of death.

Obstetrical causes of death (Table II)

Infection was the cause of maternal death in about 11% of cases and was so with the same relative frequency during both the 10-year periods despite

Table I *The maternal mortality rate during the periods studied*

	Number of deliveries	Maternal mortality	
		n	Per 100 000 deliveries
1955-64	1 083 400	710	19.3
1965-74	1 139 483	86	7.5
1955-74	2 222 909	296	13.3

Table II *Obstetrical causes of death*

Years	Infection		Pre eclampsia		Eclampsia		Haemorrhage		Thrombo-embolism		Air embolism		Amniotic embolism	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
1955-64	17	11.2	16	10.5	29	19.1	45	29.6	9	5.9	7	4.6	13	8.6
1965-74	7	10.4	7	10.4	12	17.9	12	17.9	2	3.0	1	1.5	15	22.1
1955-74	24	11.0	23	10.5	41	18.7	57	26.0	11	5.0	8	3.7	28	12.6

Table III *Non obstetrical causes of death*

Years	Appendicitis	Ileus	Anaesthesia	Heart failure	cerebral haemorrhage	Aortic aneurysm	Pneumonia	Malignancy	Suicide	Various	Total
1955-64	1	10	3	12	8	2	3	8	2	11	47
1965-74	1	2	2	2	1	3	3	2	1	7	19
1955-74	2	12	5	14	9	5	6	10	3	18	66

the fact that the absolute number of deaths from sepsis has diminished by more than 50%.

Pre eclampsia was the cause of death in 10.5% of cases; there was no difference in the relative frequency between the two periods. The decrease was only numerical even in this group.

Eclampsia was considered to be the principal cause of death in 19%; there was no relative difference between the two 10 year periods.

Haemorrhage The proportion of deaths attributable to haemorrhage was 26%; there seems to be a slight relative and numerical decrease in the second period regarding this category.

Amniotic embolism was diagnosed in 9% during the first 10-year period and in 2% during the second. The diagnosis was established histologically in 9 of the 13 cases in the first period and in 1 of 15 cases during the second period. Some of

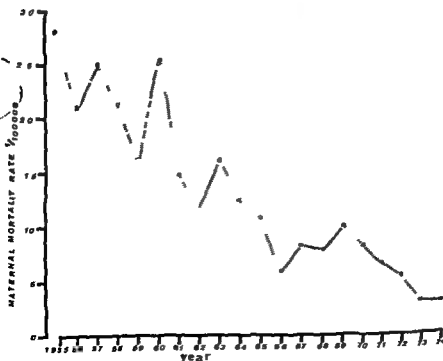


Fig 1 Maternal mortality in Sweden 1955-74

of	Inversion of uterus		Total number	
	n	%	n	%
1955	0	—	152	100
1969	1	1.5	67	100
1979	1	0.5	219	100

classified as haemorrhage during the first period might therefore have suffered from unnoticed amniotic embolism

rupture of the uterus was diagnosed in 12%. There was no significant difference between the two periods.

Obstetrical causes of death (Table III)

There has been a great variation in the cause of death, especially during the first period including accidents, suicides, virus infections etc. The difference between the two periods seems to be a slight decrease concerning ileus and heart failure.

DISCUSSION

Swedish women in Sweden give birth in hospital (over 90%). The number of maternity departments headed by specialists in obstetrics and gynaecology has increased during the last 10 years. During the periods studied 87.2% of all deliveries took place in such departments. However, there was no significant difference in the rate of maternal mortality between maternity departments headed by or not headed by specialists in obstetrics and gynaecology (Table IV). The explanation is probably

that most hospitals have had units for intensive care during the last 10-year period. The absolute decrease in maternal mortality during the last 10-year period includes all causes of death. As there has been no change in nutritional standards during the time of observation and the access to medical care has been almost the same over the last 20 years, the explanation could be higher quality of the medical care given in the hospitals during the last 10 years.

Especially the decreased number of cases of heart failure and ileus seems to prove this assumption.

REFERENCES

1. Bjerre H & Åstedt B. Maternal Mortality in Sweden. *Acta Obstet Gynecol Scand* 43: 1, 1964.
2. Brody S. Modramortalitet. *Läkartidningen* 64: 4789, 1967.
3. Furstenberg N. Maternal Mortality. A statistical study of earlier and present prognosis in childbirth. *Acta Obstet Gynecol Scand* 28: 103, 1949.
4. Sjövall A. Mutterliks mortalitet i Schweden. *Dtsch Gesundheitsw* 16: 380, 1961.

Submitted for publication March 21, 1977

Stefan Fianu
Department of Obstetrics and Gynecology
Sabbatsberg Hospital
S-11382 Stockholm
Sweden

IV Maternal mortality rate during the studied periods

Maternity departments without specialist in obst & gynec				Maternity departments headed by specialist in obst & gynec				Maternal mortality	
Maternal mortality				Maternal mortality				Total number of deliveries	Rate/100 000
Number of deliveries	n	Rate/100 000		Number of deliveries	n	Rate/100 000			
64	146 143	37	21.9	937 283	178	18.9		1 083 426	210
74	138 608	11	7.9	1 000 875	75	7.4		1 139 483	86
74	284 751	43	15.8	1 938 158	253	13.0		2 272 909	296

EFFECT OF GENERAL AND LOCAL ANAESTHESIA ON BLOOD LOSS DURING AND AFTER THERAPEUTIC ABORTION

Birger R. Møller, Jørgen Trer Hansen and Søren Møntzen

From the Surgical Out patient Clinic, Aarhus Kommunehospital, Aarhus, Denmark

The blood loss occurring during therapeutic abortion performed under local and general anaesthesia and the postoperative bleeding was measured in 60 women in the first trimester of pregnancy. They were 20-30 years old (mean age 24.3 years), all nulliparae. They were divided into three equal groups according to the duration of pregnancy. In each group 10 patients underwent abortion under general anaesthesia and the remaining 10 under local anaesthesia. In all cases cervical dilatation by the Hegar method and vacuum aspiration were used and all operations were performed by the same surgeon. General anaesthesia was induced with atropine and thiopental and maintained with a mixture of nitrous oxide and oxygen in small doses of thiopental and pethidine. Local anaesthesia consisted in paracervical blockade produced by injection of 1% lidocaine-adrenaline. The blood loss was smallest in the 7th and 8th weeks of pregnancy under both local and general anaesthesia and increased with the gestational age. In all three groups the blood loss during operation under general anaesthesia was twice as large as under local anaesthesia. There was no difference in the postoperative bleeding under general and local anaesthesia. It is concluded that local anaesthesia has several advantages. Paracervical blockade provides a rapid and reliable anaesthesia which is safe for most patients. The costs, delays and complications of general anaesthesia are avoided. Local anaesthesia is well suited for outpatients and the blood loss is reduced to a minimum.

Several studies on therapeutic abortions (14, 15, 17, 18) widely varying complication rates have been reported. A variety of techniques for the induction of pregnancy and different types of anaesthesia have been employed. During the last years nearly all authors have expressed the opinion that cervical dilatation by the method of Hegar and vacuum aspiration are safe procedures well suited for outpatient intervention.

In most series studied the operation was performed under general anaesthesia, but some re-

ports (2, 10, 13) seem to indicate that the blood loss is smaller when local anaesthesia is used. However, in these studies the operations were performed by various staff members, the blood loss was measured only during the operations and postoperative bleeding was not recorded. As in some of these studies the patients constitute a heterogeneous group as regards age, parity and duration of pregnancy, the results are difficult to compare. In the investigation presented here an attempt was made to collect a homogeneous group of patients. The blood loss was measured both during the operation and postoperatively and all the operations were performed by the same surgeon. In this way it will be possible to compare the blood losses occurring when either general or local anaesthesia is used.

MATERIAL AND METHODS

The patients studied were 60 healthy women in the first trimester of pregnancy undergoing elective therapeutic abortion. Their ages ranged from 20 to 30 years and averaged 24.3 years; all were nulliparae. The gestational age was determined clinically by the obstetrician on the basis of the menstrual history and estimation of the size of the uterus. The patients were divided into three equal groups according to the duration of pregnancy as follows: Group I: duration less than 9 weeks; Group II: 9-10 weeks; Group III: 11-17 weeks. In each group 10 patients underwent operation under general anaesthesia, while local anaesthesia was employed in the remaining 10.

Informed consent to the procedure was obtained from all the patients concerned.

In all cases the procedure was cervical dilatation by the method of Hegar and vacuum aspiration. This procedure has previously been described in detail by others (10, 16). All the operations were performed on outpatients by the same surgeon, who had wide experience in this intervention.

All the patients were premedicated with diazepam 10 mg intravenously. General anaesthesia was induced with

Table 1 Blood loss during and after therapeutic abortion in 60 first trimester pregnancies

Duration of pregnancy	Bleeding during operation (ml)				Postoperative bleeding (ml)			
	Local anaesthesia		General anaesthesia		Local anaesthesia		General anaesthesia	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Under 9 weeks	21.9	15-30	38.0	24-50	70.0	10-30	19.2	15-24
9-10 weeks	40.9	32-60	70.6	50-90	35.0	25-50	11.5	5-19
11-12 weeks	77.7	70-95	145.0	110-200	49.0	30-65	54.5	40-70

intravenous injections of atropine 0.5 mg, tubocurarine 3 mg and a small dose of thiopental 250-300 mg. Small doses of pethidine 20-40 mg were administered as an analgesic. The anaesthesia was maintained by sufficient additional thiopental. In a few cases small doses of succinylcholine and inhalation of a 2:1 mixture of nitrous oxide and oxygen.

Local anaesthesia consisted in paracervical blockade produced by 1% lidocaine-adrenaline 10+10 ml. The solution was injected into the paracervical tissue at the junction of the cervix and vagina in the lateral fornices at the 4 and 8 o'clock positions. The needle was inserted 10 mm into the tissue, the syringe was aspirated and then 10 ml of the solution was injected on either side of the cervix.

Postoperatively all patients were given methyl ergonovine maleate (Methergine®) 0.2 mg intramuscularly.

As already mentioned the blood loss was measured both during the operation and postoperatively.

Blood loss during operation

During the suction the uterine contents were collected in a container. After the operation a known amount of 5% sodium hydroxide was added and 24 hours later the optical density of the resulting alkaline haematin solution was measured. The result was compared with the patient's venous blood treated similarly. The principle of this was described by Hallberg & Nilsson (8) and modified by Kasonde & Bonnar (9). Blood loss on drapes and sponges was minimal and therefore disregarded.

Postoperative bleeding

The patient's blood loss after the operation was determined by collecting sanitary towels (Mölnlycke A/S Copenhagen) in buckets and extracting the haematin using sodium hydroxide in a known amount as mentioned above. The haemoglobin loss was calculated from the extinction of the resulting alkaline haematin solution and the volume of the extract. The postoperative bleeding was then estimated from the haemoglobin concentration and compared with the patient's venous blood treated similarly.

RESULTS

The results are summarized in Table 1. Mann-Whitney's test was used in the statistical analysis. In all three groups the blood loss during operation

under general anaesthesia was nearly two as under local anaesthesia, i.e. the difference was highly significant ($p < 0.05$).

In contrast to this the postoperative bleeding showed no statistical difference with the type of anaesthesia. In both types of anaesthesia the blood loss was smallest in the 7th and 8th pregnancy and increased with the gestational age.

Under both types of anaesthesia the operation lasted slightly more than 5 min, but never more than 10 min. The duration of general anaesthesia about 10 min and never more than 15 min.

In most cases postoperative bleeding stopped within 1-2 days; in all cases it stopped within 3 days. The procedure did not give rise to complications; in particular no clinical signs of endometritis, salpingitis or parametritis, no haemorrhages requiring blood transfusion and no uterine perforations or cervical ruptures were encountered.

DISCUSSION

Considerable blood losses may occur during therapeutic abortion performed in the first trimester of pregnancy. Dvorak et al. (5) described the problem of patients requiring transfusion after therapeutic abortion and Eaton (6) reported blood loss ranging from 100 to 500 ml during suction curettage of the pregnant uterus.

It is well documented (4, 17) that halothane is a potent uterine relaxant associated with an increase in blood loss during legal abortion. Some authors (10) have suggested that halothane is contraindicated as an anaesthetic agent for this procedure because of the enhanced blood loss associated with its use. Cyclopropane anaesthesia has also been reported to be followed by heavy bleeding. Therefore the authors therefore concluded that it should be avoided for this procedure.

he method of general anaesthesia used in this study is reported to be safe and well suited for brief surgical operations (4). Thiopental can decrease uterine contractility (7) and thus perhaps increase the blood loss. Clinical experience suggests a little inhibitory effect on uterine contractility and in one study (4) it is reported that the drug is smaller than in any other form of general anaesthesia.

The local anaesthesia used in our study was provided by lidocaine with added adrenaline. Adrenaline has a vasoconstrictive action which decreases the absorption of local anaesthetics and reduces blood flow in the tissues. One observation indicates that lidocaine is a potent vasoconstrictor when applied to the arteries in the pregnant uterus and that this agent stimulates the smooth muscles of the pregnant uterus *in vitro* (3). In addition, lidocaine seems to potentiate the stimulating effect of adrenaline (1). All these effects seem to decrease the blood loss during the operation. The difference in the blood losses during general and local anaesthesia observed in our study is in keeping with the findings just mentioned.

In conclusion it may be said that the choice of general or local anaesthesia appears to have a definite effect on the amount of blood loss during elective therapeutic abortion. We found that the blood loss under local anaesthesia was only one half of that occurring under general anaesthesia. The bleeding was well tolerated by all the patients who were young and healthy women, but the occurrence of a profuse haemorrhage may very well be hazardous to a patient with a limited cardiovascular reserve.

We find that local anaesthesia has several advantages. Paracervical blockade provides a rapid and reliable anaesthesia which is adequate for most patients. The costs, delays and complications associated with general anaesthesia are avoided. Local anaesthesia is well suited for out-patient intervention and the blood loss is reduced to a minimum.

REFERENCES

1. Astrom A. Influence of some local anesthetics upon the adrenaline contraction of isolated strips of rabbit aorta. *Acta Physiol Scand* 60: 30 1964.

2. Benic H M & Kupresanin M. Vacuum aspiration using paracervical block for legal abortion as an out patient procedure up to the 12th week of pregnancy. *Lancet* 2: 619 1971.
3. Cibils L A. Response of human uterine arteries to local anesthetics. *Am J Obstet Gynecol* 126: 702 1976.
4. Cullen H F, Margolis A J & Eger E I. The effects of anesthesia and pulmonary ventilation on blood loss during elective therapeutic abortion. *Anesthesiol* 32: 108 1970.
5. Dvorak Z, Trnka V & Vasecek R. Termination of pregnancy by vacuum aspiration. *Lancet* 2: 997 1967.
6. Eaton C J. Uterine aspiration for evacuation of the pregnant uterus. *JAMA* 207: 1887 1969.
7. Friedman E A. Effects of drugs on uterine contractility. *Anesthesiol* 26: 409 1965.
8. Hallberg L & Nilsson L. Determination of menstrual blood loss. *Scand J Clin Lab Invest* 16: 244 1964.
9. Kasonde J M & Bonnar J. Effect of sterilization on menstrual blood loss. *Br J Obstet Gynaecol* 83: 572 1976.
10. Kerslake D & Casey D. Abortion induced by means of the uterine aspirator. *Obstet Gynecol* 30: 35 1967.
11. McGaughey H S, Corey E L, Eastwood D & Thornton W N. Effect of synthetic anesthetics on the spontaneous motility of human uterine muscle *in vitro*. *Obstet Gynecol* 19: 233 1962.
12. Miller J R & Stoelung V K. Halothane in obstetric anaesthesia. *Anesthesiol* 23: 256 1965.
13. Moberg P, Sjoberg B & Wikqvist N. The hazards of vacuum aspiration in late first trimester abortions. *Acta Obstet Gynecol Scand* 54: 113 1975.
14. Møller B R, Diederich P, Hansen J T & Oram V. Abortus provocatus legalis. *Ugeskr Læg* 138: 379 1976.
15. Møller B R, Hansen J T, Diederich P & Oram V. Therapeutic abortion in an out-patient clinic. *Acta Obstet Gynecol Scand* 57: 41 1978.
16. Nathanson B N. Ambulatory abortion. Experience with 76000 cases. *New Engl J Med* 286: 403 1977.
17. Stewart G K & Goldenstein F. Medical and surgical complications of therapeutic abortions. *Obstet Gynecol* 40: 539 1972.
18. Tietze C & Lewit M. Joint program for the study of abortion (JPSA). Early medical complications of legal abortion. *Studies in Family Planning* 8: 97 1977.

Submitted for publication Nov. 3 1976

Burger R Møller
Miltonsvej 11
DK-8770 Højbjerg
Denmark

DOXYCYCLINE (VIBRAMYCIN®) IN PELVIC INFLAMMATORY DISEASE

Halvard Gjønnæss and Eirik Holten

From the Department of Gynecology and Obstetrics Aker Hospital and the Bacteriological Laboratory Aker Hospital Oslo Norway

Abstract Using standardized laparoscopy technique fluid aspirated from the pouch of Douglas from the Fallopian tubes and from ovarian cysts in 85 cases with clinical diagnosis of pelvic inflammatory disease (PID). The concentration of doxycycline in the aspirates was measured after oral ingestion of 200 mg of doxycycline (Vibramycin®). A therapeutic level was achieved in the tubes and in ovarian cysts within a few hours and on continuation of treatment these values followed the plasma concentration closely still being within the therapeutic range 24 h after the final dose. The clinical effect was excellent 94% (60/64) of the cases with verified PID being cured by doxycycline.

There are numerous microorganisms capable of causing pelvic inflammatory disease (PID). They are frequently not identified presumably due to technical difficulties and recent reports indicate a relatively high frequency of anaerobic bacteria (6). The roles of *Mycoplasma* and *Listeria* are still uncertain. Tetracyclines are often used in treatment of PID. Doxycycline (Vibramycin®) has been claimed to have certain advantages over other tetracyclines because it is so well absorbed, has a slow breakdown in serum and has a low frequency of side effects (4, 5, 9). Its antibacterial effect *in vitro* against aerobic and certain anaerobic bacteria has been found to be higher than that of other tetracyclines although the number of resistant strains of bacteria seems to be increasing (1). Beside the *in vitro* antibacterial effect the *in vivo* effect also depends upon the drug concentration at the site of infection. The crucial point in the treatment of PID is presumably the concentration of antibiotic obtainable within the lumen of the Fallopian tubes although the penetration of doxycycline into various tissues has been the subject of many investigations (8, 9, 10). We have not found any report on

investigation of the intratubal doxycycline concentration.

The aim of this study has been to determine the concentration of doxycycline in tubal fluid compared with the plasma levels to observe the time interval from oral ingestion of standardized doses until a therapeutic level of doxycycline is reached within the Fallopian tubes and the maintenance of this level after cessation of treatment.

MATERIAL AND METHODS

Diagnostic laparoscopy was performed in 85 women with a clinical diagnosis of pelvic inflammatory disease (PID). This diagnosis could not be verified in 21 cases. Twenty-two patients had ovarian cysts, 9 of them in combination with PID. The frequency of gonococci in the cervix was 18% in those cases where cervical secretion was examined bacteriologically. In cases of gonorrhoea the symptoms of PID developed after therapeutic abortion in 2 cases and postmenstrual in 3 (Table II).

On admission doxycycline treatment was initiated whenever PID was suspected with 200 mg orally followed by subsequent daily doses of 100 mg. Within 1-2 days laparoscopy was performed and using the standard technique fluid was aspirated from the Fallopian tubes and from the pouch of Douglas. The latter fluid was considered to be of tubal origin in cases of tubal patency. From hydrofoppyo-salpinges and from ovarian cysts fluid was obtained through long cannulas inserted transabdominally.

Table I Bacteriological findings in 85 patients with a clinical diagnosis of pelvic inflammatory disease

Age (years)	n	Not performed	Gonorrhoea	Non specific microbes
16-20	32	7	5	0
21-30	43	23	3	17
30-45	10	5	1	4
Total	85	35	9	41

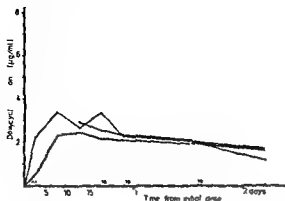


Fig 1 Doxycycline concentration in the Fallopian tubes (---) capillary blood plasma (—) and venous blood plasma (· · ·) after standard oral treatment

under visual guidance. Plasma was obtained from finger tip capillary blood. The recordings were made only once from each patient.

The concentration of doxycycline was determined using standard paper disks from AB Biodisk, Solna, Sweden, according to the method of Jalling, et al. (7). Antibiotic medium No. 1 (Difco) was used, and the test bacterium was *Bacillus cereus* ATCC 11778. Peritoneal fluid from non-treated cases had no effect on this bacterium.

Because of technical error 3 cases had to be omitted from the total series of 85.

RESULTS

Doxycycline in tubal fluid

Within 10 hours from the primary dose of doxycycline the plasma and tubal concentrations averaged 2.5 µg/ml or more (Fig. 1). Continued treatment revealed concentrations averaging 2 µg/ml. The mean tubal and plasma concentrations then

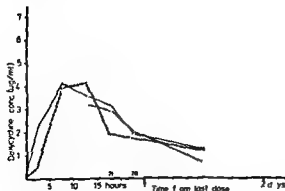


Fig 2 Doxycycline concentration in the Fallopian tubes (---) capillary blood plasma (—) and venous blood plasma (· · ·) related to the last dose of doxycycline given orally

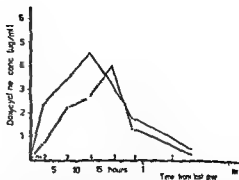


Fig 3 Doxycycline concentration in ovarian cysts and capillary blood plasma (—) related to the last dose of doxycycline given

showed identical levels throughout the observation period. Even though the mean values were identical, the individual cases did not always show a good correlation. The correlation coefficients were 0.68–0.80–0.61–0.57–0.56–0.57–0.47 for the respective time intervals in Fig. 1. The presence or absence of active inflammatory disease did not seem to influence these results. After 4 h, nearly all of the patients had received their 1st dose of 100 mg doxycycline. After the treatment was discontinued, there was a parallel and rapid decrease in tubal and plasmas concentrations (Fig. 2).

In 7 cases of tubal occlusion the doxycycline concentration was recorded in tubal and peritoneal fluid separately (Table II). These values were almost identical so that our working hypothesis—that of peritoneal fluid being representative of the tubal exudate in the case of tubal occlusion—seems justified.

Doxycycline in ovarian fluid

In 22 patients fluid was aspirated from ovarian cysts and examined for doxycycline concentrations (Fig. 3). The mean concentrations in ovarian fluid and plasma showed similar corresponding tubal and plasma levels.

Clinical effect

In 4 patients with PID, doxycycline treatment was discontinued because of doubtful or poor effect.

Table II Mean doxycycline concentrations in patients with tubal occlusion

Tubal fluid	1.3 µg/ml
Peritoneal fluid	1.4 µg/ml
Plasma	1.8 µg/ml

of them was diagnosed 3 months post partum and in one case the PID appeared after a menstrual period. In none of them were specific bacteria isolated. They all responded promptly to ampicillin. The 9 patients with gonorrhoea were all apparently cured by doxycycline. In none of the patients did the treatment have to be discontinued because of side effects.

DISCUSSION

The penetration of the antibacterial agent to the actual inflammation sites is of vital importance for its anti-infectious effect. For doxycycline the plasma, tubal and ovarian concentrations showed therapeutic levels within a few hours from the initial dose (Figs 1-3). The maximum concentrations were recorded within 10 hours after the initial dose was given. The mean doxycycline level from then showed only a minor decrease throughout the observation period, demonstrating therapeutic levels more than 24 hours after the final doxycycline dose.

The doxycycline concentrations in the tubal fluid in the majority of cases exceeded the values given for minimum inhibitory concentration against numerous aerobic and anaerobic microbes (2). The excellent clinical effect in 94% (60/64) of the cases with PID indicates that the doxycycline concentrations found in the tubes were adequate even *in vivo*. The rapid resorption of doxycycline, the high concentration and the achievement of an equilibrium between the plasma and tubal or ovarian concentrations indicate excellent penetration of the drug into these organs. Our observations are in agreement with reports dealing with serum concentrations after oral ingestion of 200 mg of doxycycline (5) and on doxycycline penetration into other organs (8-10).

ACKNOWLEDGEMENTS

We are indebted to Mrs M. Vangdal for technical assistance and also to our colleagues in the gynaecological

department who kindly participated in the collection of the material.

REFERENCES

1. Chow A W, Patten V & Guze L B. Comparative susceptibility of anaerobic bacteria to minocycline, doxycycline and tetracycline. *Antimicrob Agents Chemother* 7: 46, 1975.
2. Dornbusch M & Nord C E. In vitro activity of tetracyclines on aerobic and anaerobic bacteria. *In Opuscula Medica Suppl* 33: 21, 1974.
3. Dornbusch K, Nord C E & Wadstrom T. Biochemical characterization and in vitro determination of antibiotic susceptibility of clinical isolates of *Bacterioides fragilis*. *Scand J Infect Dis* 6: 253, 1974.
4. English A R & Lynch J M. Doxycycline: A resume of microbiological studies. *In Opuscula Medica, Suppl* 33: 11, 1974.
5. Fabre J, Piton J S & Kunz J P. Distribution and excretion of Doxycycline in Man. *Chemotherapy* 11: 73, 1966.
6. Finegold S M. Intra abdominal genito-urinary skin and soft tissue infections due to non sporeforming anaerobic bacteria. *In Infection with Non sporing Anaerobic Bacteria* (ed I Phillips & M Sussman) p. 169. Churchill Livingstone, Edinburgh, London and New York, 1974.
7. Jalling B, Malmberg A S, Lundman A & Boreus L O. Evaluation of a micro-method for determination of antibiotic concentration in plasma. *Eur J Clin Pharmacol* 4: 150, 1972.
8. Lutziger H. Konzentrationsbestimmungen und klinische Wirksamkeit von Doxycyclin (Vibramycin®) in Uterus, Adnexen und Muttermilch. *Ther Umschau* (Bern) 26: 476, 1969.
9. Otten H, Piempel M & Siegenthaler W. *Antibiotika*. Fibel 4th edn pp 345-361. Georg Thieme Verlag, Stuttgart, 1975.
10. Wetterfors J & Sjodahl R. Doxycycline concentrations in certain tissues following intravenous and oral administration. *In Opuscula Medica Suppl* 33: 64, 1974.

Submitted for publication Aug 31 1976

Halvard Gjønnæss
Kvinneklinikk
Aker Sykehus
Oslo 5
Norway

THE INFLUENCE OF LOCALLY ADMINISTERED PROSTAGLANDIN E_2 AND $F_{2\alpha}$ ON UTERINE MOTILITY IN THE INTACT NON PREGNANT HUMAN UTERUS

J N Martin Jr M Bygdeman and P Eneroth

From the Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden

Abstract. Clinical studies on the effect of locally administered prostaglandin E_2 and $F_{2\alpha}$ on the sensitivity and reactivity of the non pregnant human uterus were performed in 16 volunteers. With the use of the flaccid balloon technique, uterine recordings were made at frequent intervals throughout the menstrual cycle. As little as 0.25 μg PGE_2 or 1.0 μg $\text{PGF}_{2\alpha}$ effected an increase in uterine motility during most of the proliferative and secretory phases of the menstrual cycle. However, a marked decrease in sensitivity and suppression of reactivity to either prostaglandin compound was observed around ovulation. Moreover, an inhibition of uterine contractility in response to PGE_2 but not to $\text{PGF}_{2\alpha}$ was noted during active menstrual bleeding. Circulating levels of estrogen could be correlated generally with these uterine responses. Endogenous prostaglandins normally occur in the secretory endometrium in levels compatible with the amount of exogenously administered prostaglandins which elicited increased, decreased or unchanged uterine activity in this study. These findings suggest that local prostaglandin E_2 and $F_{2\alpha}$ in concert with variable levels of circulating estrogen may play important roles in the delicate regulation of uterine motility during the menstrual cycle.

von Euler (7) reported in 1934 that seminal fluid contains relatively high concentrations of potent smooth muscle stimulatory agents called prostaglandins. Many investigators have undertaken intensive studies in attempts to identify physiological roles for these compounds in the complex interplay of events which constitute the human reproductive process. Areas of special interest have been sperm transport, sperm capacitation, tubal function and fertilization itself. Moreover, in recent years there have been several reports investigating the myometrial response of the non pregnant human uterus to systemically administered prostaglandin E_2 and $F_{2\alpha}$ (3, 10, 15).

Attention has been focused in the regulation of uterine contractility around ovulation and during menstruation.

It is well known that the non pregnant human uterus exhibits a characteristic general pattern of spontaneous activity which varies according to the different phases of the menstrual cycle. It appears that estrogen generally exerts a predominantly stimulatory or at least facilitatory effect in contrast to the depressive myometrial effect of progesterone (4, 9, 13).

Whereas *in vitro* work has suggested that PGE compounds generally inhibit the contractility of human myometrial strips (1, 16), reports from *in vivo* work indicate that intravaginal, intramuscular or intravenous PGE cause uterine stimulation. One exception is during the concurrent intravenous administration of oxytocin and vasopressin when inhibition has been reported following vaginal administration of extract of human seminal fluid (6). Prostaglandin $F_{2\alpha}$ consistently stimulates uterine myometrium both *in vitro* and following systemic *in vivo* administration (1, 3, 10, 15, 17). The significance of uterine recordings made following the systemic administration of prostaglandin is however difficult to interpret with regard to uterine sensitivity since the amount of compound reaching the uterus is small and variable.

The present investigation was performed to study the sensitivity and reactivity of the non pregnant human uterus to the intra-uterine administration of physiological amounts of PGE_2 or $\text{PGF}_{2\alpha}$ during the various phases of the menstrual cycle and to compare these responses with circulating estrogen and progesterone levels.

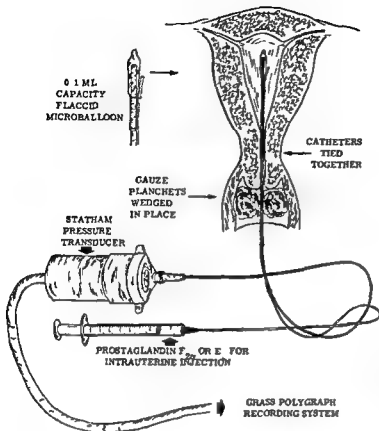


Fig 1 The figure illustrates the recording system with the flaccid microballoon. Prostaglandin $F_{2\alpha}$ and $F_{2\beta}$ were administered into the uterine cavity through a 0.66 mm polyethylene catheter tied to a recording line.

PATIENTS AND METHODS

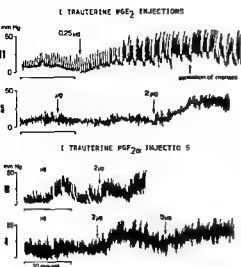
Sixteen women were chosen for this investigation. All volunteers had a history of regular menstrual cycles and were found to have normal gynecological examinations. Eleven women were nulliparous and 5 were multiparous. All contraceptive agents except condom were terminated at least one month prior to the beginning of the study. Abstinence was prohibited 24 hours before each recording session.

Ovulation was confirmed by a biphasic basal temperature chart and by a rise in serum progesterone. There were 11 recording sessions including five control sessions. All patients were recorded at least once during the proliferative and secretory portions of the cycle as well as during menstruation and around the expected time of ovulation. Six patients received only $PGF_{2\alpha}$ and 6 received only PGE_2 . 4 patients received both compounds during single recording sessions for comparison.

Uterine contractility was recorded by means of a flaccid microballoon (0.1 ml capacity made by London Rubber Ltd) tied to a polyethylene catheter and connected to a Statham pressure transducer and a Grass polygraph. The fluid filled air tight system was calibrated before each recording. At the volunteer's first visit the direction and length of the cervico-uterine canal was determined by sounding the uterus. The microballoon tipped catheter was inserted gently through the endocervical canal at least 1 cm beyond the internal os. The catheter was secured in place by wedging several gauze planchets into the vagina and around the catheter line prior to removal of the

speculum (Fig 1). The patient then gently assumed a supine position and remained quietly there for the duration of the 2-3 hour recording session. Baseline uterine activity was recorded for at least 20 minutes prior to prostaglandin instillation. The prostaglandin $F_{2\alpha}$ solution was prepared immediately before testing by diluting a stock solution (5 mg/ml) with physiological saline to achieve a concentration of 5 or 20 μg $PGF_{2\alpha}$ per ml. The PGE_2 solution was prepared by diluting concentrated stock buffered saline to achieve a concentration of either 1 or 2 μg PGE_2 per ml. Small volumes were frozen immediately and maintained at $-20^{\circ}C$ until use. At each recording session increasing amounts of the $PGF_{2\alpha}$ or PGE_2 (0.1-1.6 ml) were administered into the uterine cavity by means of a pre-filled 0.66 mm polyethylene catheter tied to the recording line. The time interval between administrations was usually allowed to return to baseline uterine activity levels. Uterine stimulation or inhibition was considered significant whenever basal tonus was elevated more than 10 mmHg or if there were an obvious change in the number and/or frequency of contractions. A single control recording was acted as control during 5 recording sessions spaced throughout the menstrual cycle. Each session was spaced up to 1.6 ml normal saline injected into the uterine cavity.

Serum for progesterone and estradiol levels were collected and stored at $-20^{\circ}C$ until analysis. Progesterone was determined with the antibodies and the procedure of Micromedex Diagnostic Inc (Progesterone



Uterine recordings showing the uterine response to PGE_2 and $PGF_{2\alpha}$ during the preovulatory phase (4–11 before ovulation)

immunoassay kit). The purity of the progesterone (Applied Science) was ascertained by thin layer chromatography as well as by determination of its molar absorptivity index (REF) in absolute alcohol. The purity of 3H labeled progesterone used for monitoring of procedural losses was checked with the chromatographic methods. Estradiol 17 β in 1.0–2.0 ml serum was determined after extraction (2 \times 10 ml ether Mallinckrodt) and chromatography on columns of Sephadex LH 20 in the solvent methanol 95/5 (v/v). 3H labelled estradiol (New England Nuclear) 5000 dpm was added to serum prior to extraction. The estradiol fraction (8.5–15.5 ml effluent) was assayed with antibodies from New England Nuclear (044). The RIA procedure recommended by the manufacturer was followed. The purity of the estradiol (Applied Science) and of 3H labelled estradiol was checked in the same way as for progesterone.

RESULTS

The non pregnant human uterus responded in a characteristic way to the intra uterine instillation of PGE_2 or $PGF_{2\alpha}$ according to the phase of the menstrual cycle. The patterns of response could be divided arbitrarily into four phases: the preovulatory, periovulatory, postovulatory and menstrual. Reference to the time of uterine recording in this paper therefore was based upon the number of days before (–) or after (+) the estimated date of ovulation.

Preovulatory phase

From the cessation of menstruation until approximately 4 days before ovulation the intra uterine instillation of small amounts of either PGE_2 or $PGF_{2\alpha}$ generally resulted in a relatively rapid stimulation of uterine contractility. The effect of injected prostaglandin upon uterine motility during this phase could be characterized by a gradual rise in uterine tonus accompanied by increased amplitude of contraction. No evidence of inhibition was observed. As little as 1.0 μg of either compound usually effected a response and stimulation was sustained or enhanced with larger doses of prostaglandin (Fig. 2). Quantitatively less PGE_2 than $PGF_{2\alpha}$ appeared to effect a definite response in those patients who received both compounds during a single recording session.

Periovulatory phase

Fig. 3 illustrates the relative lack of uterine response to the intra uterine administration of PGE_2 or $PGF_{2\alpha}$ respectively around the time of ovulation. In contrast to the previously seen stimulatory response of the preovulatory uterus, higher doses of prostaglandin around ovulation induced little change from a typically low amplitude, high frequency, regular baseline pattern. In several patients given higher doses of PGE_2 preceded or followed by single instillations of $PGF_{2\alpha}$, neither compound consistently elicited an obvious stimulatory nor inhibitory response. The period of relative non-response could extend as long as 4 days after the estimated time of ovulation, but thereafter blended into the next phase.

Postovulatory phase

Several days after ovulation until the onset of menstrual bleeding, spontaneous uterine activity under the influence of progesterone characteristically evolved into increasingly irregular, less frequent and higher amplitude contractions similar to those seen late in the prelabor phase of term pregnancy. Locally administered PGE_2 or $PGF_{2\alpha}$ during this portion of the menstrual cycle invariably resulted in stimulation. The overall stimulatory response was qualitatively and quantitatively less marked than early in the cycle. Particularly following PGE_2 injections, the stimulatory response early in the postovulatory phase was manifested by an increase in the duration and intensity of contractions while an elevation of uterine tonus was not always a part of the

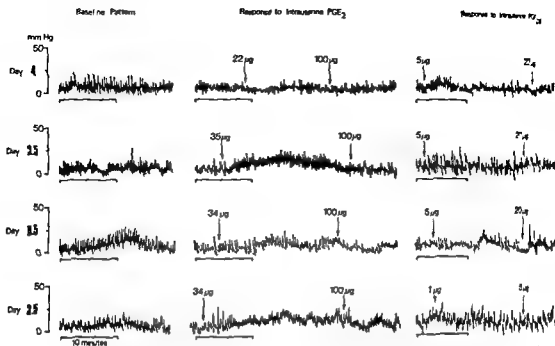


Fig 3 Relative uterine unresponsiveness to even large intra uterine instillations of PGE_2 and $PGF_{2\alpha}$ administered to the same patients during the preovulatory phase (4 days before to 31 days after ovulation)

effect. Later in this phase an increase in resting uterine tonus was noted to occur as part of the response to increasing amounts of PGE_2 and $PGF_{2\alpha}$ and this was accompanied sometimes by mild discomfort. As demonstrated in Fig 4 doses of $PGF_{2\alpha}$ equal to those of PGE_2 seem to elicit greater responses in contrast to the pattern observed during the preovulatory phase.

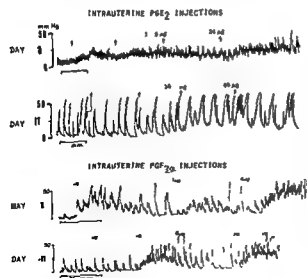


Fig 4 Uterine response during the late secretory phase (6–11 days after ovulation) to intra uterine instillations of PGE_2 and $PGF_{2\alpha}$.

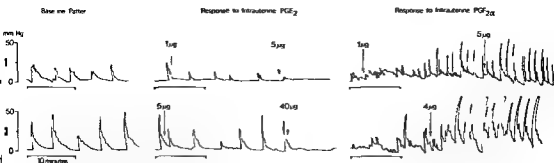
istered to the same patients during the preovulatory phase (4 days before to 31 days after ovulation).

Menstrual phase

The only inhibitory response to the intrauterine injection of prostaglandin occurred following PGE_2 instillation during menstrual bleeding. The response to $PGF_{2\alpha}$ in contrast continued to be consistently stimulatory. As shown in Fig 5 uterine reactivity was inhibited by small amounts (1–5 µg) of PGE_2 and more strongly depressed by larger doses of PGE_2 for periods lasting up to 70 min while the intrauterine injection of 1–5 µg $PGF_{2\alpha}$ elicited a more intense stimulatory response.

Hormonal levels and uterine reactivity

Based upon basal body temperature charts and blood sampling for progesterone at each recording all 16 study subjects entered the test menstrual cycles. Estrogen levels in addition to progesterone values were determined each recording session for the last 5 cycles studied and were consistent with previously reported data. The mean estrogen values are shown in Fig 6 with 95% confidence limits. The serum estrogen levels achieved during the menstrual cycle occurred just prior to ovulation and coincided in time with the period of maximal uterine reactivity to locally administered $PGF_{2\alpha}$ was most depressed. Moreover the mean levels of circulating estrogen during



5 Uterine recordings from two patients during the estradiol phase. Of note is the inhibitory effect of PGE_2 and stimulatory effect of $PGF_{2\alpha}$ in the same patient

ovulatory phase corresponded to the time of the most easily demonstrated reactions to PGE_2 and $PGF_{2\alpha}$.

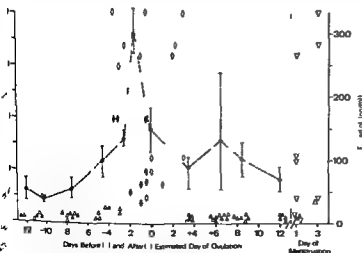
Later in the postovulatory phase during the second peak of estrogen the reactivity to both prostaglandin compounds appeared muted.

DISCUSSION

The efficacy of the flaccid microballoon technique for the recording of uterine contractility as described by several investigators has become as well established as the open end method (3, 4). The microballoon method avoids problems involving mucous secretions which can be encountered with open end catheters lacking sponge tips and is

especially more suitable than the continuous flow system when studying the effect of intra uterine administration.

Baseline variations in uterine contractility throughout the menstrual cycles of the 16 women studied were comparable to the characteristic patterns described previously by others using a variety of recording techniques (4, 9, 13). Even though all recordings exhibited features of these general patterns, a wide variation was noted between patients. A variation in uterine sensitivity to prostaglandins during the menstrual cycle *in vivo* following systemic administered prostaglandin has not been demonstrated (3, 10, 15). However, following intravenous administration both PGE_2 and $PGF_{2\alpha}$



6 Summary of threshold values for locally administered PGE_2 and $PGF_{2\alpha}$ in 16 patients during the menstrual cycle together with the mean estrogen values. 95% confidence limits for 5 patients. Threshold

stimulation dose PGE_2 ○ $PGF_{2\alpha}$ ▲ Highest dose PGE_2 ■ Highest dose $PGF_{2\alpha}$ ▼ The dose causing initial inhibition sometimes followed by stimulation PGE_2 ▼

are very rapidly transformed into biologically less active metabolites and thus the amount of the compounds eventually reaching the uterus is small and variable.

The present investigation demonstrates that the response of the non pregnant human uterus to locally administered PGE_2 and $\text{PGF}_{2\alpha}$ is dependent upon the phase of the menstrual cycle. Stimulation by both prostaglandin compounds usually an increase in uterine tonus was observed from the cessation of menstruation until approximately 4 days before ovulation. During the periovulatory phase a relative insensitivity to as much as 20 μg of intra uterine $\text{PGF}_{2\alpha}$ or 100 μg PGE_2 was noted in all study subjects.

Thereafter until the onset of menses the uterine response to E and F prostaglandin was characterized by a more tempered increase in contractional amplitude and activity than by an outright increase in uterine tonus. These results are in accordance with those we have reported earlier (12, 13). Finally during menstrual bleeding uterine contractility could be depressed by locally administered PGE_2 in contrast to the consistently stimulatory effect of $\text{PGF}_{2\alpha}$. The intra uterine instillation of physiological saline to the control patient failed to effect any change in uterine contractility at any time during the menstrual cycle.

The present results are compatible with the earlier findings by Embrey as quoted by Pickles et al (14). Embrey found that intra uterine administration of as little as 0.5 μg $\text{PGF}_{2\alpha}$ stimulated increased uterine contractility in 2 patients on days 24 and 26 of their menstrual cycles. The only *in vivo*

investigating the effect of locally administered prostaglandin E_2 upon the nonpregnant human uterus have been reported recently by Toppozada and his co workers (18, 19). These investigators also found a stimulatory response following the intrauterine instillation of PGE_2 in the pre- and postovulatory phases of the menstrual cycle. However they reported an inhibitory response to PGE_2 during the periovulatory phase in contrast to the lack of response observed in our study. Another major point of difference was the much higher doses of PGE_2 necessary to elicit uterine responses in the Toppozada studies. One reason might be the different PGE_2 preparation used.

The levels of $\text{PGF}_{2\alpha}$ as its major metabolite (15-keto-13,14-dihydro- PGF_2) in the human endometrium during the menstrual cycle has been meas-

ured recently by Green & Hagenfeldt (20) using chromatography mass spectrometry technique. A high concentration of 15-keto-13,14-dihydro- $\text{PGF}_{2\alpha}$ was found during the luteal or postovulatory portion of the cycle, little if any of the compound could be detected in the endometrium at the time of ovulation. Downie et al (9) measured the concentration of primary prostaglandins, PGE_2 and $\text{PGF}_{2\alpha}$, increased progressively during the luteal phase of the cycle. At least during the luteal portion of the cycle the endometrial concentration corresponded to the exogenous $\text{PGF}_{2\alpha}$ which elicited a uterine response in the present study.

Pickles' earlier bioassay work demonstrated that the primary prostaglandins are present in the endometrium throughout the menstrual cycle, although secretory endometrium has a higher content of $\text{PGF}_{2\alpha}$ with an increased $\text{PGF}_{2\alpha}$ to PGE_2 ratio and menstrual fluid contains about 10 times more $\text{PGF}_{2\alpha}$ than is found in secretory endometrium (14).

The present findings do suggest that $\text{PGF}_{2\alpha}$ and PGE_2 compounds may play important physiological roles in the cyclic regulation of uterine motility during the menstrual cycle. During menstruation a disturbance in the relative balance of these compounds with increased $\text{PGF}_{2\alpha}$ and/or a deficiency of PGE_2 originally suggested by Pickles may be an important factor involved in the increased myometrial activity and pain noted in patients suffering from primary dysmenorrhea, especially since treatment with prostaglandin biosynthesase inhibitors such as indomethacin reduce uterine activity and relieve as well as ease the pain for the patient (11).

Periovulatory relative insensitivity to both PGE_2 and $\text{PGF}_{2\alpha}$ corresponded to the highest level of estrogen achieved during the menstrual cycle. The highest sensitivity was before and after menstruation when the estrogen levels are low. Myometrial reactivity to prostaglandins therefore may at least partially be under hormonal control similar to what has been reported for vascular smooth muscle (21).

It has been speculated that the high prostaglandin E concentration in human seminal fluid may enhance uterine contractility after intercourse and facilitate passive sperm transport. The relatively low sensitivity of the myometrium to PGE_2 from ovulation to PGE_2 indicates that other factors may be responsible to the statistically significant correlation observed between low human

1 PGE concentration and infertility (2) To help her evaluate the role of prostaglandins in human reproductive functions additional careful studies are needed to study the interrelated effects of vaginally administered upon the human cervix, cervical os and fallopian tubes

ACKNOWLEDGEMENT

This work was supported by the WHO Expanded Programme of Research Development and Research Training

REFERENCES

Bygdeman M The effect of different prostaglandins on the motility of the human myometrium *Acta Physiol Scand* 63 Suppl 242: 1 1964
 Bygdeman M, Fredriksson M, Svanborg K et al The relation between fertility and prostaglandin content of seminal fluid in man *Fertil Steril* 21: 677 1970
 Coutinho E M & Mara H III The contractile response of the human uterus, fallopian tubes and ovaries to prostaglandins *in vitro* *Fertil Steril* 22: 539 1971
 Gasapo A J & Pinto-Dantas C R The cycle activity of the nonpregnant human uterus *Fertil Steril* 17: 34 1966
 Downing J, Poyser N L & Wunderlich M Levels of prostaglandins in human endometrium during the normal menstrual cycle *J Physiol* 236: 465 1974
 Olsson R & Posse N The effect of prostaglandin on the nonpregnant human uterus *in vitro* *Acta Obstet Gynecol Scand* 39: 112 1960
 von Euler U S Zur Kenntnis der pharmakologischen Wirkung von Nativschreien und Extrakten männlicher accessorscher Geschlechtsdrüsen Naunyn-Schmiedeberg's Arch Exp Path Pharmacol 175: 78 1934
 Green K & Hagenfeldt K Prostaglandins in the human endometrium Gas chromatographic-mass spectrometric quantitation before and after IUD insertion *Am J Obstet Gynecol* 122: 611 1975
 Hendricks C H Inherent motility patterns and response characteristics in the nonpregnant human uterus *Am J Obstet Gynecol* 96: 874 1966
 Canim S M M, Hillier K, Somers K et al The

effects of prostaglandin E_1 and $F_{2\alpha}$ administered by different routes on uterine activity and cardiovascular system in pregnant and nonpregnant women *J Obstet Gynaec Br Comm* 78: 172 1971
 11 Lundström V, Gréen K & Wijkvist N Prostaglandins, indomethacin and dysmenorrhea *Prostaglandins* 11: 893 1976
 12 Martin J N Jr & Bygdeman M The effect of locally administered PGF $_{2\alpha}$ on the contractility of the nonpregnant human uterus *in vitro* *Prostaglandins* 9: 245 1975
 13 Martin J N Jr & Bygdeman M The effect of locally administered PGE $_2$ on the contractility of the nonpregnant human uterus *in vitro* *Prostaglandins* 10: 253 1975
 14 Pickles V R, Hall W J, Best F A et al Prostaglandins in endometrium and menstrual fluid from normal and dysmenorrheic subjects *J Obstet Gynaec Br Comm* 72: 185 1965
 15 Roth Brandel U, Bygdeman M and Wijkvist N Effect of intravenous administration of prostaglandin E_1 and $F_{2\alpha}$ on the contractility of the nonpregnant human uterus *in vitro* *Acta Obstet Gynec Scand* 49 Suppl 5: 19 1970
 16 Sandberg F, Ingelman Sundberg A & Rydén G The effect of prostaglandin E_1 and E_2 on the human uterus and the fallopian tubes *in vitro* *Acta Obstet Gynecol Scand* 43: 95 1964
 17 Sandberg F, Ingelman Sundberg A & Rydén G The effect of prostaglandin $F_{1\alpha}$, F_2 , $F_{2\alpha}$ and F_3 on the human uterus and the fallopian tubes *in vitro* *Acta Obstet Gynecol Scand* 44: 585 1965
 18 Topozada M, Gaafar A & Shaala S *In vitro* inhibition of the human nonpregnant uterus by prostaglandin E_1 *Prostaglandins* 8: 401 1974
 19 Topozada M, Gaafar A, Shaala S & Osman M The relaxant property of local prostaglandin E_1 on the nonpregnant uterus—a cyclic response *Prostaglandins* 9: 475 1975

Submitted for publication March 17 1977

Mare Bygdeman
 Dept of Obstetrics and Gynecology
 Karolinska Hospital
 S-10401 Stockholm 60
 Sweden

HUMAN OVARIAN TUMOURS HETEROTRANSPLANTED TO NUDE MICE

Stig Kullander Alf Rausing and Claes Trope

From the Department of Gynaecology and Obstetrics and the Institute of Pathology University of Lund
Allmänna Sjukhuset Malmö and the Tornblad Institute University of Lund Lund

17 different human ovarian tumours were heterotransplanted subcutaneously to female nude

When small tissue pieces were used 10 out of 16 mice grew. Subcutaneous injections of suspended tumour cells were made to 11 mice; all failed. Metastatic filtrative growth was never seen in the mice observed up to 25-40 days. Material from each tumour was planted to four separate sites in two mice. The successful grafts largely retained the original morphological features. As measured with ^3H -thymidine incorporation DNA synthesis was also similar in the original tumours and the growing grafts. This animal *in vivo* model might be highly informative for the study of *inter alia* fundamental biology, chemotherapy and irradiation of ovarian tumours.

The investigation and treatment of human malignant tumours can be facilitated by a suitable experimental animal model. Previous techniques have been based on transplantation of malignant tumours to immunologically privileged sites e.g. the intracerebral site in mice, the intrasplenic site in the intraperitoneal pouch and the anterior chamber of the eye in rats and guinea pigs (4, 5) or to experimental animals immunologically deprived after thymectomy (1). However, all these tumour host systems have disadvantages. In some, total chromosomal changes shift, and in others, signs of interspecies hybridization were observed (7, 12, 15).

The potential model is the heterotransplantation of human tumours into nude mice. The nude mouse is a mutant homozygous for the recessive X-linked gene *nu*. These mice were first noted by Jackson & Cattanaach (1962). Later Pantelouris (8) found that homozygous mice are born with a thymus, giving an immunological incompetence that permits the acceptance of a wide range of allografts. Rygaard & Povlsen (1969) succeeded in transplanting a human colon adenocarcinoma to nude mice. A number of human tumours have been successfully transplanted (2, 9, 13, 16, 18, 20, 22). Chromosome analyses of tumours in early and late passages (19), isoenzymes and

immunological studies have shown that human malignant tumours transplanted to nude mice can maintain their original human properties (20).

Only occasional studies of a few ovarian tumours have been published (8). These tumours offer special interesting features because of their varying histopathology, hormone-dependency and antigenic properties. This report is concerned with the acceptance, growth and histology of a number of grafted human ovarian tumours. We wished to know whether different types of ovarian tumours were transplantable in nude mice and whether they would conserve the morphological characteristics of the original tumours. The study also included a comparison of transplantability of solid tumour fragments and cell suspensions and of the rate of DNA synthesis in the tumour tissue before and after grafting.

MATERIAL AND METHODS

5-6 weeks old female nude mice (*nu/nu*) C 57/B1/6J/Bom/*nu* were used. Originally the heterozygous mice were supplied by G. L. Bomholtgard Ltd, Ry, Denmark and later bred in our own colony. We used clean but not fully pathogen free conditions which allowed an

Table I Histological classification and number of tumours studied

Histopathology	No. of cases studied
A Epithelial cancer	
Serous	4
Mucinous	4
Endometrioid	3
Undifferentiated carcinoma	8
B Sex cord stromal tumour	
Granulosa cell	1
C Lipoid cell cancer	1
D Benign tumours	
Cystadenofibroma	1

Table II Tumour material and results of grafting

0=no tumourtake +=successful tumourtake A=ascites C=cystfluid

Case	Age of patient	Histology of tumour	Clinical stage	Previous tumour treatment	Cell suspension from solid tumour	Cells from cyst fluid or ascites	Solid tumour fragments	Original site
1	76	Serous	III	Irradiation	0	-	-	Metastases
2	44	Serous	III	Irradiation	-	0	-	Primary
3	69	Serous	III	Irradiation	0	0	-	Primary
4	68	Serous	III		0	0	(+)	Primary
5	57	Mucinous	Ia		0	-	0	
6	60	Mucinous	IIa		0	-	+	Primary
7	54	Mucinous	IIc		-	0	+	Metastases
8	61	Mucinous	III	Irradiation	0	-	0	
9	71	Endometrioid	Ia	Cytostatic drugs	0	-	0	
10	52	Endometrioid	Ic	Irradiation	0	-	-	Metastases
11	62	Endometrioid	Ic		-	-	0	
12	76	Undiff ca	IV		-	0	+	Primary
13	76	Undiff ca	IV		-	0	+	Metastases
14	64	Undiff ca	IV		-	-	+	Metastases
15	65	Undiff ca	Ib	Irradiation	0	-	0	
16	73	Undiff ca	III		-	-	-	Primary
17	56	Undiff ca	IIc		-	+	(A)	Metastases
18	60	Undiff ca	IV		-	-	+	Primary
19	57	Undiff ca	III		-	+	(C)	Metastases
20	62	Granulosa	III		0	0	+	Metastases
21	29	Lipoid	Ib		0	-	0	
22	73	Cystadenofibroma	-		-	-	(+)	Primary

average life span of 4 months. The mice were fed ordinary laboratory food and water *ad lib*. The coarse sawdust bedding was not sterilized.

Tissue fragments from ovarian tumours were obtained at laparotomy. Ascites was obtained at laparocentesis or laparotomy and cystic fluid was obtained at aspiration of ovarian cysts. Within one hour the solid tumour material

was cut into small pieces measuring 3x3x3 mm with scissors in Parker 199 (SBL Stockholm) for 10 min. In a number of cases some of the small pieces were brought into a suspension of single cells with the aid of stainless steel mesh. Cells from cystic fluid and ascites were obtained by low speed centrifugation.

The technique for incorporation of labelled nucleosides into tumour DNA *in vitro* was described in detail by Håkansson & Tropé (1973). The cell suspensions were incubated for one hour with tritiated thymidine at a final concentration of 2 MCi/ml. All tests are performed in triplicate. The cell suspensions are then washed with phosphate buffer and the cells are precipitated with cold trichloroacetic acid which extracts nucleosides and nucleotides. The amount of thymidine incorporation and the DNA content of each sample is measured and an expression is calculated to estimate the amount of incorporated thymidine per DNA in the sample. This expression has a convenient form to make statistical calculations easier.

The formula is

$$a = 100 \log \frac{(c.p.m.) \times 10^4}{(AES) \times (DNA)}$$

Where (c.p.m.) is the number of counts registered with the scintillation counter, (AES) is the amount of DNA in the sample expressed in arbitrary units but corrected with a standard curve.

Under sterile conditions two nude mice were injected subcutaneously with four pieces 2x3x3 mm of the tumour tissue and often also two mice with control

Table III Successfully grafted tumour fragments

Case	Histological differences between primary and transplanted tumours when fragmented pieces are used	Takes/Attempts	Serial transplants/takes
2	Slight	5/8	4/3
4	Great (Grafts under resorption?)	2/8	1/0
6	None	6/8	2/1
7	None (In one graft lymphoid tissue was found)	3/8	0
12	None	4/8	0
13	None-slight	4/8	0
14	None	4/8	1/0
17	None	2/8	0
18	None	4/8	3/2
19	None	3/8	0
20	None	3/8	0
22	None	1/8	0

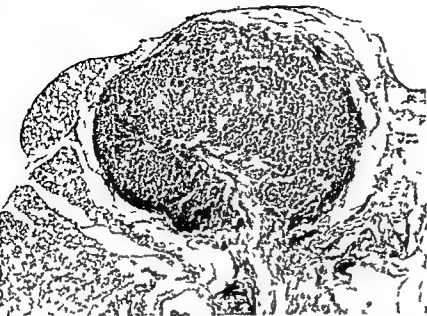


Fig. 1. Implant of poorly differentiated ovarian carcinoma (17). Thin fibrous capsule surrounding implant and septa radiating from it $\times 70$.

volume of 0.1 ml of Parker 199 (SBL, Stockholm) in cystic fluid cells or ascites cells were used. 2-6 cells were injected subcutaneously. Two pieces or injections were put on each side of the mouse over the front and hind leg regions respectively. The animals were observed daily and tumour appearance was evaluated every third day by palpation. A minimum size of $4 \times 4 \times 4$ mm was considered a sign of acceptance and growth. This period was approximately 25 to 40 days after inoculation. When the animals were killed the acceptance rate among the grafts was recorded. The tumour grafts were taken from the inoculation sites and some samples of the tumours were then brought into cell suspension for DNA synthesis determination. The remaining pieces were fixed in Bouin's solution for paraffin wax embedding, sectioned, stained with hematoxylin-eosin and microscopic examination.

The histological picture was compared with that of the original tumour tissue. In some cases the tumour graft was also transplanted to subsequent generations of the nude mice. The host sometimes died or became sick for other causes and then had to be sacrificed earlier than for DNA synthesis study and histological examination. Blood could also be collected in several of these cases. Table 1 surveys the number of human ovarian tumours grafted and their original histological picture.

RESULTS

Table 11 shows patient's age, histology, clinical course of the tumour and previous treatment, also the results of grafting.

When small tissue fragments were used 10 out of 16 human tumours grew as localized tumours with a minimum size of $4 \times 4 \times 4$ mm at one or more of the transplantation sites. In six of these ten xenografts the samples came from peritoneal metastases. Two samples from metastases did not grow (cases 1 and 10) they had received previous irradiation. In four of the ten xenografts the samples came from primary tumours. Four samples from primary tumours failed to grow (One of these patients had previously been irradiated, case 3). From a total of four previously irradiated patients only one tumour (no 2) grew after transplantation.

When cell suspensions from ascites, cyst fluid or solid tumours were used the takes were poor. Only 2 transplantations were successful (cases 17 and 19) both undifferentiated cancers and with cells from ascites or a cyst respectively. Eleven trials with cell suspensions made from solid tumour tissues were negative.

Tumour inocula could usually be recognized as established after 14 days and grew as well circumscribed tumours. The tumours were not infiltrating into the skin or underlying tissue. They could easily be observed, measured and biopsied. No metastases to lymph nodes or other organs were observed. Serial growth was possible in one serous

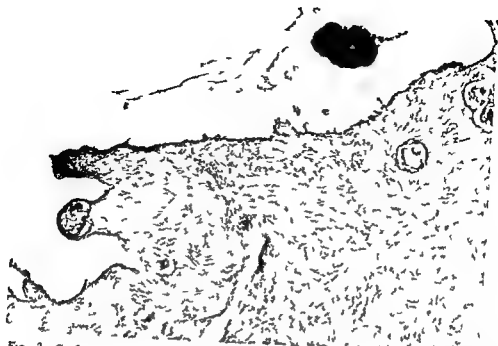


Fig 2 Graft of serous papillary cystadenocarcinoma (case 4) showing a mass of connective tissue without

tumour cells. Some calcified spherules are seen and small calcified bodies in the human tumour $\times 70$

one mucinous and one undifferentiated tumour. The serous tumour was serially transplanted for up to four passages (Table III). There were no histological differences between the primary and the serially transplanted tumours.

The microscopic picture of the grafts in all cases agreed well with the human donor tumour (Table III, Figs 3-4). In two cases (4 and 7) localized tumours were recognised; there was no histological difference between some of the

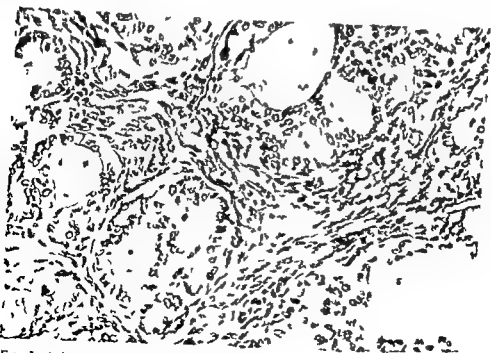
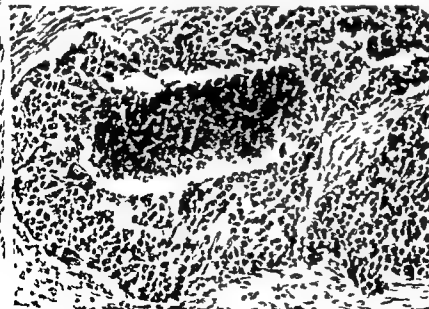


Fig 3 A human mucinous adenocarcinoma (case 7) $\times 180$



4 The same tumour as in Fig. 3 grown in the mouse

ts and the donor tumour. One (case 4) serous cystadenocarcinoma showed a mass of connective tissue without tumour cells in two of the implants. Some calcified spherules were seen similar to small calcified bodies in the human tumour. Fig. 2 shows the appearance of this tissue in the mouse. In one case of mucinous cystadenocarcinoma (no. 7) three implants grew. No tumour was found in



5 Papillary and solid adenocarcinoma in human. A mononuclear lymphocyte is seen around tumour structure (c 19) $\times 180$

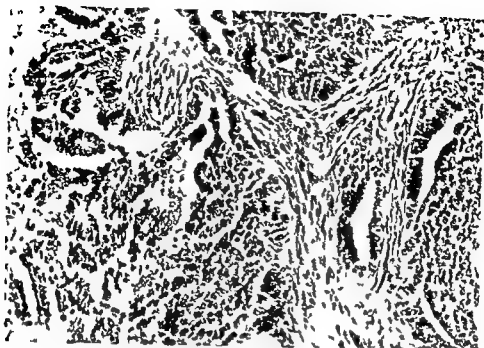


Fig 6 Implant of tumour seen in Fig 5 Lymphocyte reaction even slighter than in human $\times 180$

one only a lump of loose connective tissue with organized lymphatic tissue

The growth rate of the heterotransplanted tumour grafts was variable. One tumour (no. 4) grew very slowly and never surpassed $4 \times 4 \times 4$ mm up to the time of the animal's death. In cases 2 and 18 one

take in each showed a considerable growth measured $10 \times 10 \times 15$ mm when the animal had been killed. In case 19 spontaneous regression was observed in one out of 3 successfully grafted pieces.

In the successful implantations the grafts

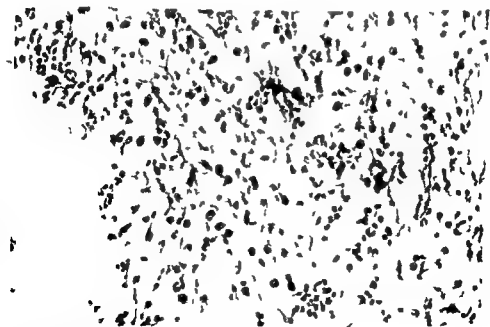


Fig 7 Diffusely growing anaplastic carcinoma of human. Aggregate of lymphocytes to the left (case 13) $\times 180$

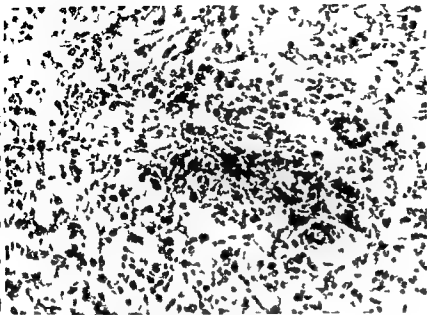


Fig. 8. Implant in mouse of tumour seen in Fig. 7. Similar histological picture and focal lymphocyte aggregate.

well-circumscribed surrounded by a condensed mass of connective tissue in all probability derived from host tissue. Fibrous septa were generally seen within the tumours. They radiated from the capsule.

(1) Thin walled vessels were seen within the tumour septa. They could often be traced to similar vessels in the capsule and were probably derived from the host. Quite extensive necrosis was often seen within parts of the implant but this was probably related to poor vascularization and further examinations of the same implant showed good vitality of the cells.

Generally there was no or only slight inflammatory reaction in the growing graft tumour tissue.

The stroma trabeculae were without reaction in cases 5 and 6 (case 19). In a diffusely growing anaplastic carcinoma (case 13) however the human tumour contained many focal aggregates of lymphocytes as did the implant in the mouse (Figs 7 and 8).

We also compared the rate of DNA synthesis in tumour tissue before and after grafting to see whether there were any differences in proliferative activity of the primary tumour and the heterotransplants. Table 4 shows the result of the incorporation studies of tritiated thymidine into DNA

In six cases (nos 2, 12, 13, 14, 17, 18) all the accepted mouse tumours had a significant DNA synthesis that is the tumours retained their proliferative capacity. There was no difference in DNA synthesis between the accepted tumours in the same animal and there was no difference between the primary human and the xenograft.

In cases 4 and 7 the primary tumours showed a significant DNA synthesis but those xenografts of these mice (4b and 7a) that histologically consisted only of a mass of connective or lymphoid tissue had no DNA synthesis. Patients 19 and 20 showed no differences in the histological pictures between the primary tumour and the xenografts but the accepted grafts did not show any DNA synthesis. In patient 6 all the tissue fragments were accepted in one mouse and all the grafts showed a DNA synthesis which did not differ from one another and did not differ from the DNA synthesis of the primary tumour cells. When transplantation with suspended cells from cyst fluid was made two grafts were accepted but neither of them had any DNA synthesis.

Fast growing mouse tumours (cases 11 and 18) showed a high ^3H thymidine uptake thus showing a high DNA synthesis and these tumours had a high



Fig 9 Serous cystadenofibroma of human (case 22) $\times 70$

acceptance rate. These were from primary human tumours.

Slow growing mouse tumours (cases 4, 20 and 22) which did not show any uptake of H^3 thymidine often came from primary tumours or

metastases with originally low uptake. The serous cystadenoma (case 22) showed practically no growth or DNA synthesis but conserved its histology; the tumour graft measured only 3×10^3 (Figs 9–10).



Fig 10 Mouse implant of tumour seen in Fig 9 $\times 70$

IV DNA synthesis in successfully grafted original tumour material and grafts

marks successful tumourtake O=marks no tumourtake DNA synthesis of the primary tumours and of the surgically transplants expressed in α values

DNA synthesis of primary tumour

Cyst cells	Ascites cells	Cell susp (solid tumour)	DNA synthesis of the transplants			
-	-	306 11 285 65	<i>a</i>		<i>b</i>	
			247 36	276 71	305 02	304 14
			347 88	276 71	341 92	341 92
			273 55	231 46	344 75	344 75
-	-	48 65 85 76	<i>a</i>		<i>b</i>	
-	46 30 75 46 (no takes)	76 48 87 66	<i>a</i>		<i>b</i>	
			75 08	49 97		
			63 36	37 51		
			27 49	17 74		
-	-	86 03 89 83	<i>a</i>		<i>b</i>	
-	-	143 25 187 34	<i>a</i>		<i>b</i>	
			198 34	117 73		
			209 58	115 03		
				108 36		
0	0	9 30 35 10	<i>a</i>		<i>b</i>	
					18 62	17 78
					16 99	22 65
						35 40
-	-	190 87 186 36	<i>a</i>		<i>b</i>	
0	123 53 174 55	145 09 136 70	<i>a</i>		<i>b</i>	
0	0	247 85 217 93	<i>a</i>		<i>b</i>	

Table IV (Cont.)

Case	DNA synthesis of primary tumour			DNA synthesis of the transplants					
	Cyst cells	Ascites cells	Cell susp (solid tumour)						
19	135 07 142 32	0	189 93 192 80	<i>a</i>			<i>b</i>		
				-	○	●	-	-	○ ●
				-	○	○	-	-	○ ●
				From cyst fluid					
20	II	II	50 32 45 22	<i>a</i>			<i>b</i>		
				-	○	●	-	-	○ ●
				-	○	○	-	-	○ ●
22	0	0	3 13 21 68	<i>a</i>			<i>b</i>		
				-	○	○	-	-	○ ●
				-	○	○	-	-	○ ○

DISCUSSION

10 out of 16 human ovarian tumours were successfully heterotransplanted when using tissue fragments. This is a very good acceptance rate. But tissue fragments from each patient were transplanted at 8 sites to 2 mice; therefore the total graft acceptance figures are not so impressive. Out of a total of 128 tissue fragments, 38 were accepted. One of our accepted grafts had a slightly different macroscopical pattern from the primary tumours. This is not surprising. The patterns within a single primary ovarian tumour can be numerous and highly different. The growth rate was different also. In one case spontaneous regression was noted.

We used the same nude mice strain as Giovanella & Stehlin (1974) but in contrast to them we did not find any sign of local invasion or metastases to lymph nodes. Also in contrast to them we succeeded in only two cases with heterotransplantation when cell suspension was used.

However Giovanella et al used human tumours that had been cultured in several passages before heterotransplantation. It is possible that when cells are cultured *in vitro* there is a selection of cell

clones with increased capacity to grow and metastasize in the nude mouse.

Preliminary LDH isoenzymes analysis of the venous blood several weeks after our grafts showed that the original human tumour picture was mirrored in the mouse blood observation supporting the view that original human tumour properties are maintained in order to further study.

Grafting of solid tissue fragments was more successful than injection of suspended cells. Tissue fragments possess their own vascular bed which has only to connect to blood vessels of the host in order to ensure survival of the graft. Blood cells have to elicit the neoformation of vessels from the surrounding tissues.

In our material all the serous tumours were low grade and high stage. Of the mucinous tumours three out of four were low stage but the first endometrioid tumours were low grade and stage. Of undifferentiated tumours 3 out of 4 were high stage, all eight being high grade. Generally high stage and high grade human tumours seem to have greater chances to be accepted as xenografts. It seems easier to succeed in heterotransplantation when using material from metastases rather than from primary tumours.

Only one of the tumours from six previously treated patients grew (no. 2). This tumour had an extremely high incorporation of thymidine in spite of the irradiation treatment which means that the tumour cells had a high proliferation rate probably increasing the possibilities for successful heterotransplantation. The other tumours in previously treated patients had a low DNA synthesis after irradiation and the cytostatic drugs might directly or indirectly have influenced cellular multiplication. DNA synthesis and the possibilities of damaged cells growing in the nude mouse. There is usually high DNA synthesis in fast growing tumours. In most cases the proliferative capacity of human tumours was maintained in the grafted grafts. Two cases (nos. 19 and 20) showed difference in the histological picture between primary tumours and xenografts but the accepted tumours did not show any DNA synthesis.

REFERENCES

- Cobb L M & Mitchley B C V. The growth of human tumours in immune deprived mice. *Europ J Cancer* 8 473 1974.
- Giovannella B C, Stehlin J S & Williams Jr L J. Heterotransplantation of human malignant tumours in nude thymusless mice. II. Malignant tumours induced by injection of cell cultures derived from human solid tumours. *J Nat Cancer Inst* 52 971 1974.
- Giovannella B C & Stehlin J S. Assessment of the malignant potential of cultured cells by injection in nude mice. In *Proceedings of the First International Workshop on Nude Mice* (ed J Rygaard and C O Povlsen) pp 279-284. Gustav Fischer Verlag Stuttgart 1974.
- Goldenberg D M. Die Verwendung einiger neuer Human Tumor Stamme in der experimentellen Krebs-Forschung. Part I. *Arch Geschwulstforsch* 29 1 1967.
- Goldenberg D M. Die Verwendung einiger neuer Human Tumor Stamme in der experimentellen Krebsforschung. Part II. *Arch Geschwulstforsch* 29 18 1967.
- Goldenberg D M & Witte S. Chemotherapy of two morphologically similar human tumours growing in the cheek pouch of the golden hamster. *H. Ad. No 1* and GW 39. *Eur J Cancer* 3 95 1967.
- Goldenberg D M, Pavia R A & Tsao M C. In vivo hybridisation of human tumour and normal hamster cells. *Nature* 250 649 1974.
- Hayashi S, Yamamoto H, Miyasawa M, Oosawa N & Ueyama Y. Heterotransplantation of human gynecological cancers to nude mice. *Excerpta Medica XIII World Congr Gynecol Obstet* 1976.
- Helsen L, Das K S & Steven I H. Human

- neuroblastoma in nude mice. *Cancer Res* 9 2594 1975.
- III Håkansson L & Tropé C. An *in vitro* study of the effect of cytostatic drugs on DNA synthesis in methyl-cholantrene induced mouse sarcomas and in rat Walker 256 tumours. *Acta Pathol Microbiol Scand [A]* 81 552 1973.
- II Isaacson A B & Cattanach C B. Two new "hairless" mutants. *Mouse News Letter* 27 31 1962.
- 12 Lampert F, von Karsch P & Goldenberg D M. Chromosomen von heterolog und homolog transplantierten Human- und Hamstertumoren. *Arch Geschwulstforsch* 32 309 1968.
- 13 Merenda C, Sordat B, Maca J P & Carrel S. Human endometrial carcinomas serially transplanted in nude mice and established in continuous cell lines. *Int J Cancer* 16 559 1975.
- 14 Pantelouris E M. Absence of thymus in a mouse mutant. *Nature* 217 370 1968.
- 15 Popescu N C, Cioloca L, Liciu F & Encut I. A study of some tumour (HR 18) and mouse tumour (HM 18) obtained by hetero-transplantation of a human melanocarcinoma. *Europ J Cancer* 6 175 1970.
- 16 Povlsen C O. Heterotransplantation of human malignant melanomas to the mouse mutant nude. *Acta Pathol Microbiol Scand [A]* 84 9 1976.
- 17 Povlsen C O & Rygaard J. Heterotransplantation of human adenocarcinomas of the colon and rectum to the mouse mutant nude. A study of nine consecutive transplantations. *Acta Pathol Microbiol Scand [A]* 79 159 1971.
- 18 Povlsen C O & Rygaard J. Heterotransplantation of human epidermoid carcinomas to the mouse mutant nude. *Acta Pathol Microbiol Scand [A]* 80 713 1972.
- 19 Povlsen C O, Visfeldt J, Rygaard J & Jensen G. Growth patterns and chromosome constitutions of human malignant tumours after long term serial transplantation in nude mice. *Acta Pathol Microbiol Scand [A]* 83 709 1975.
- 20 Povlsen C O, Fialkow R J, Klein H, Klein G, Rygaard J & Wiener F. Growth and antigenic properties of a biopsy-derived Burkitt's lymphoma in thymus less (nude) mice. *Int J Cancer* 11 30 1973.
- 21 Rygaard J & Povlsen C O. Heterotransplantation of a human malignant tumour to nude mice. *Acta Pathol Microbiol Scand* 77 758 1969.
- 22 Sordat B, Fritzsche H, Mach J P, Carrel S, Ozello L & Cerottini J C. Morphological and functional evaluation of human solid tumours serially transplanted in nude mice. In *Proceedings of the First International Workshop on Nude Mice* (ed J Rygaard and C O Povlsen) pp 269-278. Gustav Fischer Verlag Stuttgart 1974.

Submitted for publication Jan 24 1977

Claes Tropé
Department of Obstetrics and Gynecology
Malmö General Hospital
21401 Malmö
Sweden

POSTOPERATIVE IRRADIATION AND CHEMOTHERAPY IN PATIENTS WITH ADVANCED OVARIAN CANCER

C Welander K E Kjørstad and P Kolstad

From the Gynaecological Department the Norwegian Radium Hospital Oslo Norway

Abstract Two groups of patients with stage III ovarian inoma were selected for a randomized clinical trial. The first group consisted of 157 patients who had radical surgery at their first laparotomy; the second group consisted of 145 patients with inoperable disease. The aim of the trial was to compare the results of maximum external supravoltage irradiation (5000 rads) to a large abdominal field with the results of a reduced dose (3000 rads) immediately followed by chemotherapy with an alkylating agent (Thio-tepa). In none of the groups did maximum therapy prove superior to the schedule of a reduced dose combined with chemotherapy. The less costly and less toxic combined treatment was better tolerated than maximum therapy alone. Histological tumour type did not influence the prognosis in these advanced cases where spread was found outside the true pelvis.

The importance of radiotherapy in the management of ovarian malignancy is controversial (1, 3, 6, 7, 9). Increasing use of chemotherapy with promising results in many cases has also created doubt as to whether radiotherapy has a place in the treatment of advanced type of disease.

In a previous report (5) an attempt to assess the effect of preoperative irradiation in patients with inoperable disease was made. The present study examines the role of postoperative irradiation and chemotherapy for patients with advanced ovarian cancer with spread outside the true pelvis.

MATERIAL AND METHODS

In 1968-73 456 patients with malignant epithelial tumours of the ovary, stage III, were admitted to the Norwegian Radium Hospital. Of these 307 were selected for a prospective randomized study and divided in two groups. The first group hereafter referred to as the control group consisted of 157 patients who had complete removal of all visible tumour tissue at the first laparotomy. Following surgery they received 3000 rads supravoltage

irradiation to a large abdominal field 20×6 (8) cm extending from the symphysis to the T₁₂ region. The radiation was given in 18 fractions of 167 rads through parallel opposed anterior and posterior portals over approximately four weeks. The kidneys were shielded after 2000 rads. The patients were then randomized either to continue the external irradiation to a midplane abdominal dose of 5000 rads or to chemotherapy with triethylene thiophosphoramide (Thio-tepa). The initial dose schedule was 60 mg in divided doses over 6 days followed by another 60 mg administered in the same way after a rest period of two weeks. Maintenance therapy followed at lower doses usually 10-20 mg every second week. Monitoring of chemotherapy was performed by weekly or bi-weekly peripheral blood counts. Lower limits allowed were haemoglobin 10.5 g/100 ml, leucocytes $2000/\text{mm}^3$, platelets $100000/\text{mm}^3$. Kidney and liver function tests were taken before therapy started and later at monthly intervals. A high protein diet, anabolic steroids, vitamins and if indicated blood transfusions were given during treatment.

The second selected group referred to as the study group consisted of 145 patients with inoperable disease. This diagnosis was based on either the findings at laparotomy (96 patients) or on clinical examination (49 patients). They received 3000 rads midplane supravoltage radiotherapy in the manner described above before a second look or primary operation was attempted.

Approximately one third of the patients responded well to radiotherapy and could have a considerable bulk of tumour resected. In another third parts of the primary and metastases could be removed and the last third had completely inoperable disease despite the irradiation given (5). Irrespective of the outcome of the post irradiation surgery the whole "study group" was then randomized to receive another 2000 rads to the same field as before or to have chemotherapy as outlined above.

The distribution by histological tumour type is given in Table I. Survival curves have been estimated according to the life table method and no patient was lost to follow up. Separate survival curves were made for the five main histological tumour types but curves for the mesonephroid and undifferentiated tumours are omitted in the figures presented here as they were identical to the curves for the serous and endometrioid tumours.

Table I

	No	Tumour type							
		Serous		Endometroid		Mucinous		Other	
		No	%	No	%	No	%	No	%
Control group	157	84	54	26	17	28	18	19	11
Study group	145	84	58	26	18	8	6	27	18
Total	302	168	56	52	17	36	12	46	15

RESULTS

The 5 year survival for the total series of 302 patients was 16% (Fig 1). In Fig 2 the survival curves for the two groups are shown. The difference in 5 year survival reflects only the bias in selection of the two groups. Patients in whom it was possible to remove all macroscopic tumour tissue at the first laparotomy (control group) had a much better prognosis than patients with inoperable disease.

No observable difference in survival was seen between patients that had a full course of radiotherapy up to 5000 rads compared with those that had only 3000 rads followed by chemotherapy (Figs 3 and 4). At all points in the graphs the standard errors overlap. This applies to both the study group and control group. 72 patients in the study group had no postoperative radiotherapy but chemotherapy instead. The relation between histological tumour type and survival is shown in Figs 5 and 6. No differences were found between the tumours of different histology and the meso-epithelial and undifferentiated tumours followed the curves for the serous type. (Not shown in the graphs.) As shown in Table I the majority of the mucinous tumours (28/36) belonged to the con-

trol group. This may indicate that these tumours are more resectable even when spread has occurred outside the pelvis.

DISCUSSION

The prognosis for patients with advanced ovarian malignancies has changed little in the last 20 years. In an attempt to achieve better treatment results both pre- and postoperative radiotherapy has been widely used (2, 4, 6, 10, 11, 12). In spite of new techniques and advanced equipment which makes it possible to deliver a high and even distributed dose, the outlook for patients with disease outside the true pelvis is very poor. Some tumours obviously react to radiotherapy, but the problem of giving adequate doses in the abdomen arises as the necessary shielding of the liver and kidneys will substantially limit the effects of irradiation. Very few patients that are left with recurrent metastases outside the pelvis will survive for more than 2-3 years, and half of them will be dead in the first year of follow up.

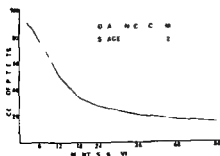


Fig 1 Survival curve for the whole group of 302 patients

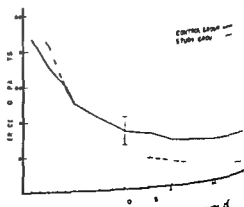


Fig 2 Survival curves for the two groups of patients studied

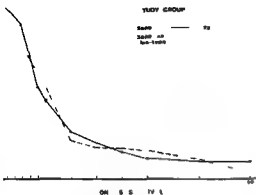


Fig 4 Survival in the study group 73 patients with a full dose of radiotherapy to 5000 rads no chemotherapy 72 patients with 3000 rads preoperative radiotherapy no preoperative radiotherapy but chemotherapy

The present study was undertaken to compare results of maximum postoperative radiotherapy with those achieved by a combination of a reduced dose of irradiation combined with immediate chemotherapy. We had two reasons for combining therapy and chemotherapy. Firstly, in our experience preoperative radiotherapy makes it possible to remove both the primary tumour and metastases in patients with inoperable disease in approximately one third of the cases (5). Therefore, it is wrong to deny patients with a poor prognosis the possible benefit of this kind of treatment. Secondly, the necessary shielding when a large abdominal field is given greatly diminishes the dose to the usual sites of spread and the additional

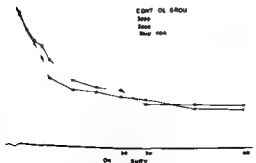


Fig 5 Survival in the control group 77 patients with a full dose of postoperative radiotherapy to 5000 rads no chemotherapy 80 patients with 3000 rads postoperative radiotherapy followed by chemotherapy

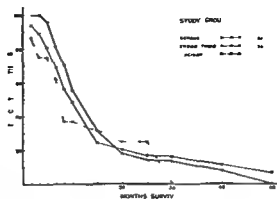


Fig 6 Survival in relation to tumour type Study group

use of chemotherapeutic agents seemed logical. The results of the present study show that maximum radiotherapy is not superior to a reduced dose of irradiation combined with an alkylating agent such as Thio-tepa. This combination therapy saves time and eliminates many of the risks and inconveniences of radiotherapy. In some centers radiotherapy in advanced ovarian cancer has been abandoned and treatment is based on single or multiple drug therapy (1-9). The 5 year survival rates achieved in the present study compare well with such treatment protocols. The degree of palliation is harder to assess as each schedule has different side effects. The patients tolerated well external irradiation to a so-called large abdominal field up to 3000 rads. In many cases ascites was reduced and the general condition of the patients improved. In the group receiving 5000 rads the frequency of severe side effects was high with loss of weight, electrolyte disturbances due to vomiting and diarrhea and lowering of serum proteins.

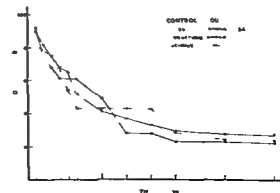


Fig 7 Survival in relation to tumour type Control group

Some authors feel that when the malignant process has spread outside the pelvis histological tumour types and degree of differentiation assume little prognostic significance (4-8). This agrees with the findings in the present study. In our opinion the sites and extent of spread are of great importance. In the future it will be necessary to reach an agreement upon a subdivision of stage III ovarian carcinomas.

REFERENCES

- 1 Barber H R K & Lwon T H Current status of the treatment of gynecologic cancer by site. *Cancer* 38: 610 1976
- 2 Delclos L & Quinlan E J Malignant tumors of the ovary managed with postoperative megavoltage irradiation. *Radiology* 93: 659 1969
- 3 Griffiths C J, Grogan R H & Hall T C Advanced ovarian cancer. Primary treatment with surgery, radiotherapy and chemotherapy. *Cancer* 29: 1 1972
- 4 Hanks G E & Bagshaw M A Megavoltage radiation therapy and lymphangiography in ovarian cancer. *Radiology* 93: 649 1969
- 5 Kjørstad K E, Welander C & Kolstad P Preoperative irradiation in Stage III Carcinoma of the Ovary. *Acta Gynecol Obstet Scand* 56: 449 1977
- 6 Kottmeier H L Ovarian cancer: response to radiotherapy. *Amer J Roentgenol* 111: 417 1971
- 7 Munnell E W The changing progress of treatment in cancer of the ovary. *Am J Obstet Gynec* 100: 790 1968
- 8 Nieminen U & Puroila E Stage and prognosis in ovarian cystadenocarcinomas. *Acta Gynecol Scand* 49: 49 1970
- 9 Smith J F, Rutledge F & Wharton J T Radiotherapy of ovarian cancer. *Cancer* 30: 144 1971
- 10 Underwood P B, Merritt J O, Latta M F, Wallace K M & Marks R D Carcinoma of the ovary. The adjunctive use of irradiation. *Gynecol Oncol* 3: 298 1975
- 11 Vaeth J M & Buschke F J The role of preoperative irradiation in treatment of carcinoma of the ovary. *Am J Obstet Gynecol* 108: 878 1970
- 12 Villasanta U & Bloedorn F G Operation, irradiation, radioactive isotopes and chemotherapy in the treatment of metastatic ovarian malignancies. *Obstet Gynecol* 10: 531 1968

Submitted for publication Feb 27 1977

K E Kjørstad
Department of Gynecology
The Norwegian Radium Hospital
Montebello
Oslo
Norway

RELAPAROTOMY IN ADVANCED OVARIAN CARCINOMA

E. Frick, J. E. Johnsson, T. Landberg and M. Snorráðottir

From the Gynecologic Section, Department of Oncology, and the Department of Pathology, University Hospital, Lund, Sweden

Abstract Patients with Stage III ovarian carcinoma were treated either with extensive initial surgery and postoperative radiation therapy (17 patients) or with a less extensive initial laparotomy, postoperative radiation therapy and relaparotomy (19 patients). Better results were obtained in the patients in the latter group.

Surgery is the chief form of treatment used in ovarian carcinoma. Bilateral salpingo-oophorectomy and hysterectomy are the standard procedure utilized by the surgeon. Varying opinions exist, however, as to how radical the surgical procedures should be in patients having advanced tumors involving the peritoneum, large vessels and intestine. Some authors recommend radical surgery in order to remove as much of the tumour as possible (3, 9). Others, on the contrary, stress the risk of dissemination of the tumour if the procedure is in exposing large tumour masses, and they do not recommend relaparotomy after radiotherapy if possible, and/or chemotherapy (5, 6, 7, 11, 12).

This article discusses the value of relaparotomy in Stage III ovarian carcinoma.

MATERIAL AND METHODS

A total of 721 patients with ovarian carcinoma were referred to the clinic during the years 1971-73. Staging was done using the FIGO classification (14) (Table I).

Treatment principles

In primary operations were as a rule performed in the gynecological departments in the receiving area. After diagnostic curettage, surgery was performed if it was not contra-indicated by the patient's general condition. The standard surgical procedure used was bilateral salpingo-oophorectomy and total hysterectomy. In non-advanced operations the uterus was not removed, however.

Infracolic omentectomy was mainly performed only in cases with apparent or suspected omental metastases.

Fertile patients with mucinous cystadenocarcinoma limited to one ovary and without tumour growth on the surface were treated with salpingo-oophorectomy only on the side involved, and with a wedge resection of the remaining macroscopically healthy ovary. When microscopy showed only unilateral disease, no further treatment was given.

Radiotherapy was started 1-3 weeks after surgery. External radiation was administered to one abdominal and one dorsal field. Irradiation parameters: 180 kV, HVL 1 mm Cu, SSD 70-80 cm, field size about 600 cm². Treatment was as a rule started with the dorsal beam, one fraction a day, with a peak absorbed dose of approximately 4 Gy (400 rad) per fraction to a total of 20 Gy (2000 rad). Three weeks after conclusion of the first series, the second series to the abdominal field was started, with a daily peak absorbed dose of 3 Gy (300 rad) up to a total of 20 Gy (2000 rad). The midabdominal absorbed dose for external therapy was usually about 12-15 Gy (1200-1500 rad).

Supplementary intracavitary treatment was given once or twice in most cases in connection with the external treatments. A rod shaped applicator (7 mm × 72 mm, 5.6 GBq equivalent to 150 mCi ²²⁶Ra) was placed in the uterus for 6-12 hours, or a flat applicator (5 mm × 44 mm × 44 mm, 4.2 GBq equivalent to 110 mCi ²²⁶Ra) was placed in the upper part of the vagina for 13-14 hours in patients having had a hysterectomy.

It was not possible to use the standard surgical procedure in all of the Stage II and Stage III ovarian carcinoma patients. Only an exploratory laparotomy was performed in some of them, and in some cases it was not possible to remove all the demonstrable tumour. If the tumour progressed in such patients, or remained unchanged during 2 months observation following radiotherapy, relaparotomy was performed at the Gynecological Section of the Department of Oncology, except in those cases where this measure was contra-indicated by the patient's general condition.

The day after relaparotomy had been performed, Melphalan was infused intravenously for 6-8 hours (1 mg/kg body weight to a maximum of 60 mg). Three to four infusions were given at 4 week intervals, and if by

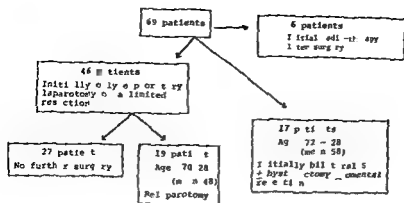


Fig 1 Treatment scheme for carcinoma Stage III

then there was any evident tumour progression no further chemotherapy was given. The interval was either wise increased to 8 weeks during the following year and in 12 weeks the second year following surgery. During the ensuing years chemotherapy was given two-to four times a year.

In patients who had not had a relaparotomy recurrences and/or metastases after the conclusion of radiotherapy were treated with Mefphalan using the same dose age described above. No further chemotherapy was as a rule given when Mefphalan therapy failed.

The distribution of patients with Stage III ovarian carcinoma is shown in Fig 1. Nineteen patients had a relaparotomy after a limited initial surgical procedure whereas 17 patients were initially submitted to a rather extensive operation. The results of therapy in these two groups will be analysed.

The original histological specimens of the above mentioned 36 patients have been re-evaluated. The distribution of histological types among patients in the two groups is presented in Table II.

RESULTS

The relaparotomy in the 19 patients was performed at a mean of 4.6 (range 4-9) months following initial

surgery. In 9 cases only an exploration was possible whereas in 10 patients the adhesion was thereof could be removed and two of these a hysterectomy. In 7 cases all demonstrable tumours could be removed and in one case only a tumour was left.

The results of therapy (NED=No Evidence of Disease and respectively deceased of carcinoma) are shown in Table III for patients initially given extensive surgery and for patients undergoing a relaparotomy after limited initial surgery. Results are calculated from the time of operation. Of 19 patients having had a relaparotomy after limited initial surgery 8 are alive NED whereas 11 have died from ovarian carcinoma. Of 17 patients initially given extensive surgery 10 are alive NED and 7 have died from the disease. Both of these groups of patients have the same mean observation time.

DISCUSSION

Laparotomy should be performed if not contraindicated in patients with suspected ovarian carcinoma even if presurgical staging indicates that the tumour may not be resectable. Five per cent of the patients who undergo a laparotomy biopsy will often establish a cancer diagnosis prior to the operation and also often the extent of the tumour. (4) Cytology cannot as a rule be relied upon for determination of the response to radiotherapy and chemotherapy. Some patients may not benefit from presurgical radiotherapy and chemotherapy and laparotomy should be performed as soon as possible. For patients with tumours judged prior to surgery as not being resectable may well prove to be so at operation.

The type of operation that should be performed

Table I Ovarian carcinoma 1971-73

Stage	Number of patients	Per cent of total
IA	49	22
IB	11	5
IC	1	<1
IIA	11	5
IIB	14	11
III	69	31
IV	24	11
Unoperated	31	14
Total	221	

Table II Distribution of histological types among patients in different treatment groups

	19 patients relaparotomy after limited primary surgery	17 patients primarily given exten- sive surgery
serous papillary adenocarcinoma and cystadenocarcinoma	9	10
viscous adenocarcinoma and cystadenocarcinoma	1	3
viscous cystadenoma of borderline malignancy	1	0
endometrioid carcinoma	1	0
clear cell adenocarcinoma	0	1
mixed epithelial tumour (mucinous + serous adenocarcinoma)	0	1
differentiated carcinoma	3	2
classified epithelial tumours	4	0

Advanced ovarian carcinoma has been the subject of much debate. Apparently no radical operation can be performed when there are widespread peritoneal metastases in the pelvis and the abdomen or when there is a large fixed pelvic tumour. Only a biopsy can then be possible. Radiotherapy and chemotherapy can on the other hand only be expected to produce lasting remission if at the most only minimal neoplastic tissue is left (1, 2, 6, 7, 11, 13). More extensive techniques have been described by Gordon & Chir (1973) where an extraperitoneal mobilization and extirpation of the pelvic contents were employed and even large tumours could be totally excised. The tumour is however often adherent to the large vessels and transecting these in an attempt to excise as much tumour as possible does not seem to be warranted. There may be great difficulties with hemostasis and the risk of massive dissemination of viable tumour cells is possible which may convert the procedure into an exercise in tumour dissemination (7). To avoid this situation only a biopsy should be performed in cases where the tumour is apparently not radically resectable. This was the case in two-thirds of the 69 Stage III patients in the present series. The postoperative radiotherapy given in the present series has been more or less the same for patients who initially only had an exploratory operation and for those who had a more extensive procedure.

Relaparotomy was performed as a standard procedure in all cases where the tumour did not progress or where the condition of the patient did not worsen during radiotherapy. The aim of relaparotomy was in all cases to remove remaining tumour.

Relaparotomy was considered to be indicated in more than one third of the patients who had initially only had an exploration. In about one half of these patients it proved to be possible to remove all or major parts of the tumour at relaparotomy.

The radiation absorbed from the dose administered is rather small due to tissue tolerance but in several cases has resulted in a marked reduction of the tumour as has also been reported from other centres (5, 6, 7, 10). A similar effect has been reported for chemotherapy by Smith et al. (8).

The further clinical course of the patients who had had relaparotomy showed that in the 8 patients in whom all or almost all demonstrable tumour had been removed no evidence of disease could be shown at the end of the follow up period 11-61 months after initial surgery. This result is better than that obtained in the group of patients where all or almost all tumour was removed at the initial operation. The two groups are however not strictly comparable. The patients undergoing relaparotomy were younger which usually implies a better prognosis in ovarian carcinoma and furthermore started chemotherapy immediately after the second operation whereas such therapy was not given to the other patients until recurrence or metastases had appeared.

The distribution of histological diagnoses also differed slightly in the two groups.

The results may indicate that in advanced ovarian carcinoma chemotherapy should be started immediately after postoperative radiotherapy in

Table III Treatment results for operated ovarian carcinoma Stage III

	Initial limited surgery + relapa- rotomy (19 patients)	Initial extensive surgery no relapa- rotomy (17 patients)
Alive NED after	8 pats 11-61 (38) months	2 pats 38-40 months
Deceased after	11 pats 5-53 (0) months	12 pats 3-50 (71) months

stead of waiting until the residual tumour has grown to detectable size

CONCLUSIONS

Surgery is the main therapy for ovarian carcinoma. As much tumour as possible should be removed but a demand for a radical procedure should not force the surgeon to transect large tumour masses since that may result in tumour dissemination. In such cases only a biopsy should be performed and postoperative radiotherapy should then be given. Such therapy will often result in marked tumour reduction in size and in some patients any remaining tumour can be successfully removed at re-laparotomy. The present study indicates that such combined therapy may be of value. The role of chemotherapy in this treatment situation has not yet been fully clarified and is the object for further investigation.

REFERENCES

- 1 Aure J C, Hoeg M & Kolstad P. Clinical and histologic studies of ovarian carcinoma. Long term follow up of 990 cases. *Obstet Gynecol* 37: 1 1971
- 2 Barber H R K & Kwon T H. Current status of the treatment of gynecologic cancer by site. *Ovary Cancer* 38: 610 1976
- 3 Hudson C N & Chir M. Surgical treatment of ovarian cancer. *Gynecologic Oncology* 1: 370 1973
- 4 Hjellegren O, Ångström T, Bergman F & Wiklund B. Fine needle aspiration biopsy in diagnosis and classification of ovarian carcinoma. *Cancer* 28: 967 1971
- 5 Kottmeier H L. Ovarian cancer with special regard to radiotherapy. *Amer J Roentgenol Radium Ther Nucl Med* 111: 417 1971

- 6 Long R T L. Recent trends in the management of advanced ovarian carcinoma. *Front Radiat Oncol* 5: 251 1970
- 7 Long R T L, Johnsson R E & Sala J W. Variations in survival among patients with carcinoma of the ovary. Analysis of 253 cases according to type, anatomical stage and method of treatment. *Cancer* 20: 1195 1967
- 8 Smith J P, Delgado G & Rutledge F. Second look operation in ovarian carcinoma. *Proc Am Soc Cancer* 38: 1418 1976
- 9 Symmonds R. Some surgical aspects of gynecologic cancer. *Cancer* 36: 649 1975
- 10 Tepper H, Sanfilippo L J, Gray J & Rosen J. Second look surgery after radiation therapy in advanced stages of cancer of the ovary. *Am J Roentgenol Radium Ther Nucl Med* 111: 711 1971
- 11 Tobias J S & Griffiths C T. Management of ovarian carcinoma. Current concepts and prospects (first of two parts). *New Engl J Med* 294: 818 1976
- 12 Wallach R C, Kabakow B, Jerez E & Brink C. The importance of second look surgery in predicting the staging and treatment of ovarian carcinoma. *Seminars in Oncology* 2: 443 1974
- 13 Wharton J T. Principles of surgical and radiation treatment for carcinoma of the ovary. In: *Current Oncology* (ed F Rutledge) p 177. J B Lippincott Sons Inc. New York 1976
- 14 Classification and staging of malignant tumours of the female pelvis. Accepted by the General Assembly of FIGO in New York on April 1st 1970. *Acta Obstet Gynecol Scand* 503: 1 1971

Submitted for publication Feb 24 1978

G Frick
Dept of Oncology
Gynecologic Section
University Hospital
S-22185 Lund
Sweden

BIPOLAR CAUTERY FOR LAPAROSCOPIC STERILIZATION

Enk Gregersen and Jens Jørgen Kjær

*From the Department of Obstetrics and Gynecology Gentofte Hospital
Copenhagen Denmark*

tract. Laparoscopic sterilization with a bipolar electrode was performed in 62 patients. The sterilization failed in one patient and was questionable in another. The effectiveness was assessed by hysterosalpingography (HSG) in 51 patients. No complications due to laparoscopy occurred. The method is considered to be safe because of the low risk of complications. The effectiveness of the method seems to be acceptable.

Laparoscopic sterilization is usually performed by coagulation with a single electrode placed in a loop and with a ground plate placed on the buttock of the patient causing the current to pass through the patient as described in Fig. 1A. However, the method is associated with a certain risk of creating unplanned sites, especially bowel and abdominal wall (2-14). If the contact between patient and groundplate is inadequate, the current becomes erratic and may pass through the operator or the anaesthetic equipment. However, intimate knowledge of the instruments and routine in the operation can reduce the frequency of complications (5, 6, 8, 10). Nevertheless, despite all precautions, complications do occur. The use of a bipolar electrode (4, 12, 13) can also serve to reduce the complications rate on account of the short circuit (1B). We have tested the method and the results are shown below.

MATERIAL AND METHOD

During the period 17.10.1975-25.2.1976, laparoscopic sterilization was performed in 67 unselected patients. For the operation Karl Storz's laparoscope and accessory equipment were used. Double incision technique was used: incision along the lower border of the umbilicus and a few centimetres above the symphysis. Sterilization was performed with the bipolar electrode 4 cm from the uterine cornu. There was no transec-

tion of the fallopian tubes. An Erbotom F2 was used as current source.

When legal abortion was to be performed, this procedure was done first.

In the puerperium, sterilization was performed between the 3rd and the 5th day post partum. Shortly before the operation, 1 ml of oxytocin was administered intravenously to obtain maximum contraction of the uterus. Because of the large uterus, the lower incision was placed a few centimetres below the umbilicus. For the same reason, the patients were not placed in the head-down position and special care was taken.

Curettage was performed if the sterilization took place in the secretory phase of the endometrium in order to exclude pregnancy.

The patients were advised to use contraceptives until control hysterosalpingography was performed at least 3 months after the operation.

The period of observation varied between 9 and 12 months.

To allow for possible change of mind, a waiting time of at least 3 months from receiving the admission papers to the admission was endeavoured; however, the waiting time was often prolonged because of the number of admissions. An endeavour was made to avoid sterilization and legal abortion simultaneously, as an unwanted pregnancy can influence the decision concerning sterilization. The patients were fully informed about the method and were told that the resulting tubal occlusion was considered to be irreversible.

RESULTS

The age distribution of the 62 patients is shown in Table 1. The youngest was 25 and the oldest 43 years. The mean age was 33.3.

The average number of pregnancies was 3.5 (0-9 abortions and 2-6 births). Maximum was 9 pregnancies in the same patient (5 births and 4 abortions).

The operation was usually performed in less than 30 minutes. The patients were without symptoms shortly afterwards and usually they were dis-

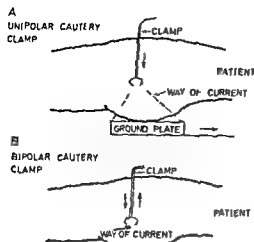


Fig 1 The two types of cautery

charged the following day so that the average hospital admission was 3.1 days. Four of our patients had another simultaneous operation (3 conizations and one vaginal repair).

No immediate complications occurred. In one case the uterus was accidentally perforated at curettage. No bleeding was seen through the laparoscope. She stayed one extra day in the department and no further problems were observed. Another patient had fever (38°C) for one day without focal symptoms. No antibiotic treatment was given and the cause remained obscure.

Four patients were sterilized on the 3rd and 4th day of the puerperium.

Legal abortion was simultaneously performed in 2 patients. Both of them were 35 years old. One had used various contraceptives without success resulting in 7 pregnancies. The other patient had been waiting for a long time for the operation.

Postoperative hysterosalpingography was performed in 51 patients (82.3%). 50 had occluded fallopian tubes. In a 37-year-old patient a small fistula at the site of the cauterization on the right side was revealed by HSG performed 16 weeks after the operation. HSG repeated 7 months later showed a larger spillage into the peritoneum. This patient was offered a new sterilization but because of lack of ability to metabolize curare she refused to undergo a further procedure. As an alternative she was recommended to use conventional contraception. As she had not used contraception in the interval between the hysterosalpingographies the risk of pregnancy was supposedly low.

In one patient legal abortion was performed in the

Table 1 Age of patients

Age (years)	Number
<25	0
25-29	13
30-34	23
35-39	23
≥40	3
Total	63

8th week of pregnancy 10 weeks after the sterilization. At the sterilization this patient had a conization performed too and thus seemed to have conceived in the following cycle. The patient had started contraception subsequently. HSG examination 7 weeks later showed occluded tubes.

HSG was not performed in 11 patients. 6 failed to attend in spite of three appointments. One patient did not want HSG performed. In 1 patient HSG was impossible because of poor conization and in another patient HSG was performed because of a premalignant lesion before HSG was available. At the operation of sterilization was found to be successful. One patient was operated on for ectopic pregnancy 11 weeks after the sterilization. At the operation the tube seemed to be closed but it was removed in order to make the occlusion definitive beyond doubt. She had not used the recommended contraception.

No pregnancies occurred later than 3 months after the sterilization. HSG was performed in 12 patients 10-11 weeks after the sterilization. In the remaining patients HSG was performed 2 weeks or more.

Prior to HSG the patients were seen in the patient clinic. The scars were hardly visible and were appreciated by the patients.

DISCUSSION

The advantages of the bipolar electrode are limited current flow, the low voltage and the reduction of uncontrolled spread of the current to a small cauterized area, which may be of importance for restoration of tubal patency. It is recommended frequently (4, 12, 13).

No laparoscopic complications occurred in our material.

Double cauterization without cutting the tube performed. This was found effective by Jordan (7). In our series one tubal fistula was found at the site of cautery. This may arise with all methods of sterilization. There may be a risk of ectopic pregnancy (15) making resterilization advisable. HSG reveals no risk of artificially opening the tube (14) which may have occurred in the above mentioned case. For this reason it is not advisable to use routine HSG as a control of the success of sterilization unless a low injection pressure is used (15). A defined observation period may be used instead (9).

The efficiency of the bipolar electrode seems to be the same as with the unipolar electrode (3, 7). The number of failures in laparoscopic sterilizations is estimated to be 0.1–2% (11). The most common reason for failure is that the woman is already pregnant at the time of the operation. Therefore, all patients in the present series had curettage performed if they were in the luteal phase. Another reason for failure is inadequate contraceptive protection before the fibrosis at the cauterized sites had time to occur. This seems to take about 3 months (1, 3, 7). This was the reason why the patients were advised to use contraceptives for 3 months or more (until the result of HSG was available). Two patients who had not used contraception received during the first 3 months. The pregnancies could not possibly have been present at the time of sterilization.

The method has one significant disadvantage—it does not offer immediate sterility. This drawback is inherent in unipolar sterilization. It is concluded that this method is preferable to unipolar electrode cautery since the efficiency is acceptable and about the same as that of the unipolar electrode and because it has several advantages.

REFERENCES

- Black W P Sterilization by laparoscopic tubal electrocoagulation. An assessment. *Am J Obstet Gynecol* 111 979 1971
- Devantier M & Larsen J Falck Komplikationer ved elektrokoagulation under laparoskopisk sterilisation. *Ugeskr Læg* 136 1790 1974
- Gregersen E & Weberg E Laparoskopisk sterilisation. *Ugeskr Læg* 136 1287 1974
- Hirsch H A & Roos E Laparoskopische Tubensterilisation mit einer neuen Bikoagulationszange. *Geburtsh Frauenheilk* 34 340 1974
- Hirsch H A Verbrennungen bei der laparoskopischen Tubensterilisation und Möglichkeiten ihrer Vermeidung. *Geburtsh Frauenheilk* 34 345 1974
- Hvidt U El. kirurgi komplikationer. *Ugeskr Læg* 136 1308 1974
- Jordan J A Edwards R L Pearson J & Mackery P J K Laparoscopic sterilization and follow up by hysterosalpingogram. *J Obstet Gynaecol-Br Comm* 78 460 1971
- Levinson C J Laparoscopy is easy—except for the complications. A review with suggestions. *J Reprod Med* 13 187 1974
- Nilsen P A & Jerve F Tubal sterilization. *Acta Obstet Gynecol Scand* 55 349 1976
- Palmer R Safety in laparoscopy. *J Reprod Med* 13 1 1974
- Population report. Series C-D number 1—1973. Sterilization. Department of Medical and Public Affairs. The George Washington University Medical Center Washington
- Roux J E & Cloutier D A new bipolar instrument for laparoscopic tubal sterilization. *Am J Obstet Gynecol* 119 737 1974
- Roux J E & Cloutier D Bipolar cautery for sterilization by laparoscopy. *J Reprod Med* 13 6 1974
- Schwimmer W B Electrosurgical burn injuries during laparoscopy sterilization. Treatment and prevention. *Obstet Gynecol* 44 576 1974
- Sheikh H H Hysterosalpingographic follow-up of laparoscopic sterilization. *Am J Obstet Gynecol* 126 181 1976

Submitted for publication Jan 1 1977

Erik Gregersen
Jørgasvej 15
DK 7100 Vejle
Denmark

AN ABDOMINAL APPROACH TO THE SURGICAL REPAIR OF POST HYSTERECTOMY VAGINAL INVERSION

□ □ Kaskarelis

*From the 1st Obstetric and Gynaecological Clinic
Athens University Alexandra Maternity Hospital Greece*

The repair of total prolapse of the vagina following total abdominal or vaginal hysterectomy is one of the problems of gynaecology.

Conservative management by use of various types of pessary has not only given disappointing results but even caused inflammation and ulceration which may occasionally result in carcinoma in situ. Furthermore in women who continue to have an active sexual life marked psychosomatic disturbances may be produced. It follows that the only treatment of total prolapse of the vagina is the surgical repositioning of the vagina in its physiological position. For this reason many techniques using either an abdominal or vaginal approach have been advocated. These techniques are technically intricate and sometimes have unpleasant complications for the patients. Furthermore the operations using the vaginal approach cause contraction of the vagina leading to complete aphareunia.

For these reasons we thought of applying a tech-

nique which will as far as possible be simple and devoid of complications and will not interfere with the patient's sexual life allowing the free performance of intercourse.

Following the routine preoperative preparations and under general anaesthesia the vagina is replaced in its physiological position. After this the abdominal wall is opened and the intestines are packed upwards with abdominal packs.

Subsequently as indicated in Fig. 2 the vaginal stump is grasped by toothed forceps and three long interrupted sutures are placed in the stump using silk (Figs 3-4). The separate ends of each suture are brought one by one to the right and left of the abdominal wall, piercing the peritoneum, the rectus abdominis and the rectus sheath 4-5 cm above the symphysis pubis (Figs 5-6). They are left free, the ends being held by Kocher's forceps. This is followed by closure of the abdominal wall up to the stage where the sheath of the rectus abdominis is



Fig. 1 Complete vaginal prolapse after total hysterectomy.



Fig. 2 Complete vaginal prolapse. The abdominal wall has been opened. The vaginal stump is grasped and drawn up by toothed forceps.

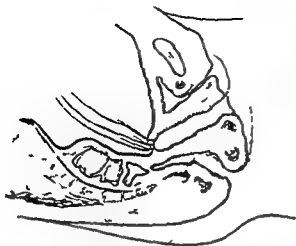


Fig 3 Three long interrupted silk sutures are placed in the stump as shown on this diagram



Fig 6 The same stage on the operating table



Fig 4 Abdominal view of the same operative stage as in Fig 3

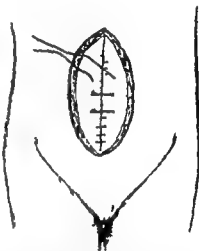


Fig 7 Traction is applied to the stump which is fixed to the abdominal wall. After the muscles have been sutured except for the skin the forceps are removed by one and the sutures are tied with multiple knots.

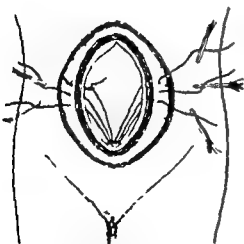


Fig 5 The ends of each suture are brought to the right and left of the abdominal incision piercing the peritoneum, rectus abdominis and rectus sheath 4 to 5 cm above symphysis pubis. The ends are left free being held by toothed forceps.

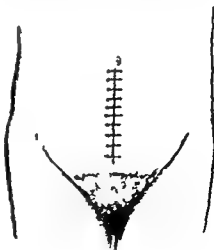


Fig 8 The skin is sutured



9 10 The post-operative results show complete appearance of the vaginal prolapse

red The forceps are then removed one at a and the ends of the sutures are pulled upwards are tied with multiple knots (Fig 7) The skin is n sutured (Fig 8)

during the course of the operation adhesions found connecting the vaginal stump to the ntum or intestines or if the vaginal stump has ered to the bladder or the rectum these are d before insertion of the sutures In cases of ole posterior colporrhaphy should be per ned

le have performed the operation in eight cases se operated on were aged 50 to 57 years

1 11 cases except in one in which we used ofilament polyamide and the suture tore ough the tissues postoperative recovery was pletely uneventful without any bladder com ations

The patients were followed up for 1-3 years and all were found to be well and without complaints The vagina was found to be in the same location as that established during the operation (Figs 9 10) and sexual intercourse was being performed fully and without pain

These findings indicate that this surgical technique is simple easy safe and without any of the complications associated with the urinary bladder which one might have expected It is furthermore possible for this operation to be performed by any gynaecologist with limited surgical experience

Submitted for publication February 27 1977

D B Kaskarelis
1st Clinic of Obst and Gyn
University of Athens
Greece

CASE REPORTS

DECREASED FETAL MOVEMENTS AND POLYHYDRAMNIOS

E Sadovsky and M Perlman

From the Department of Obstetrics and Gynecology and Neonatal Unit Hadassah University Hospital Jerusalem Israel

It was previously demonstrated that in high risk pregnancies pronounced reduction of fetal movements cessation with audible fetal heart sounds indicates fetal distress and impending death. Immediate delivery is indicated provided the fetus is viable. Three instances of cessation of fetal movements associated with polyhydramnios are reported. The fetuses proved to have malformations lethal for the newborn infant. These cases emphasize the importance of investigating fetal morphology in this condition. Antenatal diagnosis of congenital anomalies may influence decisions about intervening in cases of decreased fetal movements.

There has been increasing interest recently in the evaluation of fetal activity as an indicator of fetal well-being and distress and a number of authors have suggested that daily recording of fetal movements (FM) may be useful in monitoring high risk pregnancy (1, 2, 3). In high risk pregnancy pronounced reduction followed by cessation of FM although fetal heart sounds are still audible has been referred to as the movement alarm signal (MAS) which may indicate severe distress and impending death (3, 4). MAS may possibly serve as an indication for immediate delivery provided the fetus is viable in terms of maturity. On the basis of the following case reports it is suggested that the interpretation of diminished FM to cessation in polyhydramnios may be evaluated differently.

CASE REPORTS

Case 1 D. E. aged 24 years was admitted in the 36th week of first pregnancy due to abdominal pain, vomiting and fever for several days. On examination the uterus was found to be enlarged and the smaller fetal parts were not palpable. Polyhydramnios was diagnosed. The above symptoms continued for a week and despite the intravenous administration of fluids, maternal ketonuria and exhausted supervened. Fetal movements decreased gradually during this period and ceased for more than 12 hours. Caesarean section was performed and excessive amniotic fluid was noted on opening the uterus. A 7.5 kg female fetus was delivered with microcephaly, encephalomenia,

gocele and respiratory distress and she died 10 min after birth. At autopsy the only finding apart from the cerebral anomaly was hypoplasia of the lungs.

Case 2 P. S. aged 24 years married to a first cousin was gravida V para III. She was admitted in the thirty second week of pregnancy because of polyhydramnios. She reported that during the present pregnancy movements were fewer than in her previous gestations and that she had felt no movements for the past week. Fetal heart sounds were normal. It was decided not to induce labor although the MAS indicated fetal distress. Three days later after premature rupture of membranes and vaginal bleeding a 1.15 kg stillborn infant was delivered spontaneously. The placenta weighed 500 g and showed signs of abruption. Examination of the fetus including radiological skeletal survey established a diagnosis of achondrogenesis.

Case 3 B. S. aged 31 years gravida III para I was admitted in the thirty sixth week for severe long standing polyhydramnios. Fetal movements were within normal limits but one week later they diminished to cessation for twelve hours. Fetal heart tones were normal. A day later the fetal heart was inaudible and a week later a macerated hydropic male fetus weighing 3.2 kg was delivered spontaneously. Autopsy revealed anomalies of the alimentary tract as well as a large cystic lesion of the left adrenal gland.

DISCUSSION

Polyhydramnios is associated with congenital anomalies in more than 25% of the cases and with perinatal death in about 50% (5). Anomalies vary from lethal cranial malformations such as anencephalus (as in case 1) to correctable obstructive lesions of the fetal alimentary tract (possibly represented by case 3). But a significant proportion of cases of polyhydramnios are unexplained and unassociated with fetal and neonatal mortality and morbidity. Long standing diminutions of FM in polyhydramnios may reflect either an abnormal motility of the fetus associated with the underlying anomaly or a dampening of FM by the increased volume of amniotic fluid. Disappearance of FM preceded fetal death in cases 2 and 3 analogous to the similar natural history of diminishing FM in the fetus of high risk pregnancies (4).

The above observations indicate that one should be cautious when evaluating diminished FM in pregnancy complicated by hydramnios. The co-existence of diminished FM and polyhydramnios is highly suggestive of fetal malformation as shown by full investigation of fetal morphology as well as evaluation of the feto-placental unit. In this situation the performance of fetal radiography, ultrasonography and/or fetography and examination of α fetoprotein in the amniotic fluid may be helpful in deciding about pregnancy intervention.

REFERENCES

- 1 Mathews D D Measuring placental function Br Med J 1 439 1972
- 2 Sadosky E & Yaffe H Daily fetal movement recording and fetal prognosis Obstet Gynecol 41 845 1973

- 3 Pearson J F & Weaver J H Fetal size, growth and well being An evaluation Br Med J 1 184 1972
- 4 Sadosky E, Yaffe H & Polak I H 24 hour fetal movement monitoring in normal and abnormal pregnancy J Gynecol Obstet 12 75 1974
- 5 Hellman L M & Pritchard J A Textbook of obstetrics 14th ed p 599 Appleton-Century-Crofts New York 1971

Submitted for publication April 30 1977

E Sadosky
Department of Obstetrics and Gynecology
Hadassah University Hospital
Em Karem
Jerusalem
Israel

ACUTE FATTY LIVER OF PREGNANCY WITH DISSEMINATED INTRAVASCULAR COAGULATION

Per Moldin and Olle Johansson

From the Department of Obstetrics and Gynecology Central Hospital Borås Sweden

Abstract A patient with acute fatty liver of pregnancy associated with disseminated intravascular coagulation (DIC) is reported. The case lends support to the hypothesis that DIC may be of pathophysiologic significance in this disorder. Treatment principles are discussed and the importance of prompt Caesarean section as early as possible after the onset of symptoms is emphasized.

Disseminated intravascular coagulation (DIC) has been reported associated with complications of pregnancy for instance in abruptio placentae, toxemia, missed abortion, saline abortion, amniotic fluid embolism, post partum haemorrhage and septic abortion. Recently an association between DIC and acute fatty liver of pregnancy has been observed (3-7). It is normal in pregnancy for a number of coagulation factor levels to increase: factors I, II, VII, VIII, IX and X, and the fibrinolytic activity of the blood is reduced (1, 9, 11). Edt et al (14) have demonstrated decreased fibrinolytic activity in vein walls during pregnancy. These changes it is suggested, contribute to the onset of DIC during pregnancy. There is a divergence of opinions concerning the treatment of DIC in pregnancy. Despite different regimes, high mortality rates are reported. The intention of the following report is to document a case with acute fatty liver of pregnancy with evidence of DIC during the third trimester.

CASE REPORT

A 29-year-old primipara whose last menstrual period was in 1975 had previously had a cholecystectomy at the age of 17 years. During the 8th week of gestation vomiting and epinuria occurred but these symptoms soon subsided. Serum creatinine remained normal. Oedema appeared during the 32nd week and Chlorthiazid (Chlotride®) 0.5 g/day was prescribed. In spite of this therapy her weight

increased by 5 kg during the following 11 days. During the 36th week she was admitted to hospital after 4 days of nausea, vomiting, abdominal pain and slight generalized itching. She had lost 3.5 kg in weight during these 4 days. On admission to the hospital slight oedema was evident in the face and hands. The initial blood pressure was 170/80 mmHg, temperature was normal and foetal heart rate was 140/min. Electrolytes were normal on the following morning except for a raised serum creatinine (764 µmol/l). Serum creatinine increased during the following 2 days in spite of intravenous fluid therapy and a urinary output of 900-1 650 ml per day (Table I). Vomiting continued and on the second day she became somnolent and jaundiced with a serum total bilirubin of 183 µmol/l (Table I). Foetal heart rate remained normal. Because of her deteriorating condition the pregnancy was successfully terminated by Caesarean section under general anaesthesia. Grossly meconium stained amniotic fluid was observed during the operation but bleeding was strikingly small. 500 ml dextran (Macroder®) was given as a routine during the operation. A few hours after the Caesarean section the presence of DIC with secondary fibrinolysis was suggested by thrombocytopenia, prolonged activated partial thromboplastin time (APTT), fibrinogen/fibrin degradation products more than 40 mg/l (Wellcome Thrombo-Wellcotest), hypofibrinogenemia and signs of fibrinolysis in the blood (Table II). Treatment was initiated with dextran, fresh blood, fresh plasma and vitamin K. No heparin was given after consultation with an expert on coagulation defects. Clinical improvement commenced 74 hours after delivery with declining somnolence and oedema. Urinary output was high and urinary osmolality low (170 mmol/l). The eyegrounds were normal. During the 2 days following the operation there was a transitory reduction of arterial oxygen tension (Table I). Pulmonary X ray revealed a discrete central patchy opacity in the lungs. Serum total bilirubin reached a maximum of 213 µmol/l on the fourth day after delivery (Table I). Au antigen was negative. No bleeding tendency was observed postoperatively. The placenta was normal on histological examination. The patient was discharged home 2 weeks after delivery. At a later assessment she was clinically healthy and had normal biochemical values.

The baby's Apgar score was 10 one and five minutes after birth. Birth weight 2 830 g. Because of respiratory

Table I

	Hospital day							
	1	2	3	4	5	6	7	8
Caesarean section								
S Creatinine ($<115 \mu\text{mol/l}$)	264	380	400	410	90	100	110	121
S F protein (60–80 g/l)	62	58	45	57	59	60	55	
S Sodium (138–148 mmol/l)	140	134	130	141		140	147	
S Bilirubin (3.0–20 $\mu\text{mol/l}$)		88	85	136		168	113	180
S Al P (1.3–5.0 $\mu\text{kat/l}$)		24	20			16	13	13
S ASAT ($<0.70 \mu\text{kat/l}$)		3.4	2.6					
S Al AT ($<0.70 \mu\text{kat/l}$)		5.5	3.9					
uHb pO ₂ (10–13 kPa)			15.0	9.9	8.3	10.5	10.7	
Urinary output (ml/day)	900	1 650	4 550	5 700		1 575	1 940	2 140

distress and tachypnoea treatment with continuous positive airway pressure was instituted for two days. Antibiotic therapy was given. The child had a normal platelet count and was discharged from hospital 18 days after birth in good condition.

DISCUSSION

This patient presented clinical symptoms and laboratory abnormalities consistent with DIC with secondary fibrinolysis. Thus there were symptoms and signs of functional disturbance relating to a number of different organs: liver insufficiency with jaundice, renal insufficiency with elevated level of serum creatinine, cerebral disturbance with somnolence, abdominal pain and vomiting, placentar insufficiency with meconium stained amniotic fluid and pulmonary insufficiency with reduced arterial oxygen tension and an abnormal X-ray picture. Biochemically thrombocytopenia, hypo-

fibrinogenemia, fibrinolysis, fibrinogen degradation products in serum and a prothrombin time were documented. A reduced level of haptoglobin in serum indicated some hemolysis which was caused by intravascular coagulation (9).

The similarity of the presenting symptoms to that which is classically described for fatty liver of pregnancy was striking. The syndrome was first reported by Sheehan and called obstetric acute yellow atrophy (11). The cause is still unknown but the syndrome only occurs in pregnancy. A typical and specific liver picture is seen. The disease usually begins in the first trimester with a sudden onset of nausea and vomiting, epigastric pain, progressive jaundice, fever and sometimes haematemesis. Acute renal failure with oliguria is also part of this syndrome. The prognostic outlook is grave and maternal and perinatal mortality rates of 85% are reported (12).

Table II

	Hospital day							
	1	2	3	4	5	6	7	8
Caesarean section								
B Hemoglobin (170–160 g/l)	162	147	128	140	171	118	119	134
Leukocytes ($4\cdot9\times 10^9/l$)			11.1	17.7		11.9		
Platelet count ($100\text{--}400\times 10^9/l$)			5.31	105	98	16	68	86
P APTT (<45 s)			80	70	75	87	70	71
P TT (70–110 s)			77	17	15	11	19	16
S FDI (<10 mg/l)			>40		>40			
B Fibrinogen			sign reduced					
Fibrinolysis			sign increased					

B Fibrinogen and test for fibrinolysis according to Sharp & Fagleton (J Clin Path 16: 451, 1963)

ently an association between DIC and acute fatty liver of pregnancy has been observed (3-7). The patient also presented clinical symptoms and laboratory abnormalities compatible with DIC. This observation lends support to the hypothesis that abnormal coagulation may be of pathological importance for the development of clinical symptoms and signs in acute fatty liver of pregnancy.

Therapeutically Haemmerli (6) points out that the best chance for both mother and child is Caesarean section as early as possible after the onset of symptoms of acute fatty liver. In accordance with these observations of an association between DIC and acute fatty liver of pregnancy this therapy still seems to be the most appropriate. Most authors agree that in cases with DIC the underlying disorder should be treated primarily (8-9). DIC is a sign rather than a disease and is treated by removing the cause. In acute fatty liver of pregnancy it is the pregnancy itself which is to be considered as the cause. Interruption of pregnancy as soon as possible after the onset of symptoms therefore seems to be the therapy of choice. In the described case this treatment was successful for both mother and child. Heparin treatment has been suggested (3-7, 10) considering the mainly prophylactic effect of heparin. It has to be given early in the course of the disease to have a beneficial effect. There is also an obvious risk of serious bleeding with heparin administration especially after surgery. This patient presented manifest abnormal function associated with a number of organs before DIC was diagnosed. As there was also a risk of postoperative bleeding heparin treatment was ruled out after consultation with an expert in coagulation defects. Postoperative treatment was given with dextran, fresh blood, fresh plasma and vitamin K in order to further formation of microthrombi and to compensate for the deficit of platelets, fibrinogen and coagulation factors.

ACKNOWLEDGEMENT

We wish to thank Prof. Inga Marie Nilsson, Coagulation Laboratory, Allmänna Sjukhuset, Malmö for valuable guidance and criticism.

REFERENCES

- 1 Beller F W et al. Disseminated intravascular coagulation in pregnancy. *Clin Obstet Gynecol* 17: 250, 1974.
- 2 Beller F K. Disseminated intravascular coagulation and consumption coagulopathy in obstetrics. *Obstet Gynecol Ann* 1974.
- 3 Cano R I et al. Acute fatty liver of pregnancy. *JAMA* 231: 159, 1975.
- 4 Conaster D G & Harris R M. Fatty liver of pregnancy. *JAMA* 232: 1125, 1975.
- 5 Dixon R E. Disseminated intravascular coagulation: A paradox of thrombosis and haemorrhage. Significance in obstetrics and gynecology. A review. *Obstet Gynecol Surv* 28(6): 385, 1973.
- 6 Haemmerli U P. Jaundice during pregnancy. *Acta Med Scand* 179: Suppl. 444, 1966.
- 7 Holzbach R T. Acute fatty liver of pregnancy with disseminated intravascular coagulation. *Obstet Gynecol* 43: 740, 1974.
- 8 Kazmier F J et al. Treatment of intravascular coagulation and fibrinolysis (ICF) syndromes. *Mayo Clin Proc* 49(9): 665, 1974.
- 9 Nilsson I M. In *Blodnings- och trombosjukdomar*. Almqvist & Wiksell, 1971.
- 10 Rake M O et al. Early and intensive therapy of intravascular coagulation in acute liver failure. *Lancet* ii: 1115, 1971.
- 11 Reid D E et al. Hypercoagulable states in pregnancy. *Am J Obstet Gynecol* 111: 493, 1974.
- 12 Sheehan H L. The pathology of acute yellow atrophy and delayed chloroform poisoning. *J Obstet Gynecol Br Commonw* 47: 49, 1940.
- 13 Simpson J M et al. Intravascular coagulation and plasma fibrinogen in pregnancy. *Bibl Anat* 12: 6, 1973.
- 14 Åstedt B et al. Fibrinolytic activity of veins during pregnancy. *Acta Obstet Gynecol Scand* 49: 171, 1970.

Submitted for publication Jan. 31, 1977

Olle Johansson
Department of Obstetrics and Gynecology
Centrallasarettet
S-501 15 Borås
Sweden

DELIVERY COMPLICATED BY MYASTHENIA GRAVIS
AND EPILEPSY

U Hansson L Irestedt and P J Moberg

*From the Departments of Obstetrics and Gynecology and Anaesthesiology
Karolinska Sjukhuset Stockholm Sweden*

Abstract The literature on the possible risk of myasthenia is complicating pregnancy and delivery is sparse and y contradictory but some of the reports on the ber of pennatal and neonatal deaths are alarming psy in pregnancy implies an approximately twofold of intervention in connection with labour A pregnant nt with myasthenia gravis and epilepsy has recently delivered The case is reported and the considera with regard to suitable anaesthesia and the two dis s are discussed

are few published reports of Myasthenia vis (MG) associated with pregnancy and deliv (10 11 14 15) and of Epilepsy (Ep) as a poten risk in pregnancy (1 8) As far as can be de ined there is no report of a combination of e illnesses complicating pregnancy-delivery gh one has described complications of the penum (13) A pregnant patient with MG and has recently been treated at the department of tetics and Gynecology Karolinska Sjukhuset kholm and a report is given

CASE REPORT

patient aged 35 was primigravida She had had Ep pathic grand mal) since the age of 15 years and MG 31 years of age Her Ep was well controlled with tyoin (Diphydan®) 0.1 gram four times daily and her was controlled by pyridostigmin bromide (Mestinon®) ig three times daily as a previous thymectomy had ided no relief During the pregnancy she was given ks regularly in the hospital including control of her mphenyoin The pregnancy progressed normally and was delivered in the 39th week by caesarean section rmed under general anaesthesia and managed in the wing way
o premedication was given Preoperatively she was ed in left lateral position and was hydrated by means n intravenous infusion of 500 ml acetated Ringers tion ECG was monitored continuously Prior to in

duction of general anaesthesia 100 ml prilocaine (Citanest® 2.5 mg/ml) was infiltrated subcutaneously below the umbilicus on both sides of the midline The patient also received 0.5 mg atropine i.v. General anaesthesia was induced by spontaneous inhalation of nitrous oxide in oxygen (60-70%) and halothane (Fluothane®) was added in an increasing concentration to a maximum of 3% immediately before intubation which was then easily performed without the aid of any muscle relaxant Halothane administration was then stopped and methoxyflu rane (Penthrane® 0.2-0.6%) was added to the nitrous oxide-oxygen mixture After intubation the patient was moderately hyperventilated by manual positive pressure ventilation The induction-delivery interval was 10 minutes The course of the anaesthesia was uneventful and after completion of the operation the patient quickly re covered consciousness regained adequate spontaneous ventilation and was extubated She was observed in the postoperative ward the following 12 hours without showing any signs of complications such as muscular weakness or impaired ventilation No medical postoperative anal getics were required The child was in good condition at delivery with an Apgar score of 8 at one minute and 10 at 5 minutes and was observed in the neonatal department for 3 days without any signs of neonatal myasthenia appearing The postoperative recovery was uneventful and 6 weeks after delivery there had been no exacerbation of the MG or Ep

DISCUSSION

Influence of MG

Because of the sparse and to some extent con troversial literature relating to MG and pregnan cy-delivery (4 7) it is difficult to establish a clear cut pattern of the complications particularly those due to MG A woman with a known and well controlled MG can experience a normal pregnancy and a vaginal delivery perhaps instrumentally aided (4 10 14) Caesarean section should be used according to usual obstetric indications (14) Miller

et al (11) point out the risk of (a) prolonged labour and (b) a potential maternal and fetal danger of MG in 5 patients with 7 pregnancies there was one in partum fetal death and one postpartum maternal death. Some authors however have said that labour may be shorter than expected because of relaxation of voluntary muscles (14). McNall & Jafarnia (10) reported 5 patients who had a total of 9 pregnancies ending in one stillbirth and 7 live born babies of whom 2 died in the immediate neonatal period. Hay (7) has published a very thorough review of the literature relating to MG and pregnancy and in addition has reported on further 6 patients concluding that the combination must be regarded as a serious threat to life. Out of 9 pregnancies there were two maternal and three perinatal deaths. These reports thus support the view expressed by Miller et al (11) that MG associated with pregnancy is a potential danger. To obtain pain relief during labour the use of a regional block is advisable although small doses of local anaesthetics are recommended (2, 6). Sedatives and narcotics should be used with care due to the potential risk of increasing muscular weakness (6, 7, 10). If emergency operations such as forceps delivery or caesarian section have to be carried out a regional block can serve the purpose but might be too time consuming. In such cases general anaesthesia must be chosen which is usually well tolerated by these patients but muscle relaxants should be avoided as far as possible (2, 6, 12). General anaesthesia can then be induced by an inhalation anaesthetic as these patients are usually easily intubated in a light plane of anaesthesia (2). Induction by inhalation is slow however compared with intravenous induction combined with muscle relaxants. A planned caesarian section is thus obviously preferable to an acute one. By combining inhalation anaesthesia with infiltration of a local anaesthetic agent in the operating field the plane of anaesthesia can be kept superficial in order to avoid postoperative respiratory depression.

Influence of Ep

In a study of 371 pregnancies in women with Ep Bjerkedal et al (1) showed that complications during labour occurred with a significantly greater frequency than in non-epileptic women: 19.4% vs 5.1% vs 9.6% in controls. Intervention during labour was also significantly more frequent in patients with epilepsy.

Influence of MG and Ep

In the present case the risk of complications during labour was more than double because of Ep and the risk to the infant due to the MG was increased. Prior to labour it was necessary to discuss the methods which would be suitable for analgesia and anaesthesia in the event of vacuum or forceps extraction or caesarian section becoming necessary. The combination of MG and Ep greatly influences the choice of suitable anaesthesia. There is a contra-indication to employing a regional anaesthetic if vaginal delivery is planned but in the case of an acute complication general anaesthesia is a method of choice because of reasons mentioned above. Furthermore because of their predominantly convulsive effect local anaesthetics drugs should be used in restricted amounts in patients with Ep. When considering a planned caesarian section general anaesthesia seems to be the choice. Hyperventilation could easily occur during an epidural block or a subarachnoid block and increase the risk of a convulsive episode. Therefore large doses of local anaesthetics might interfere with neuromuscular transmission thereby constituting a relative contra-indication to epidural block. The method of general anaesthesia used in this case deserves some discussion. Halothane is an excellent agent for induction but its action on myocardial contractility is well known (3). In order to overcome uterine inertia the anaesthesia was continued with methoxyflurane in a low dose immediately after intubation. The new inhalation anaesthetic, enflurane (Efrane[®]) might have been considered as the sole inhalation anaesthetic for the whole operation but it has been shown that this drug can cause cerebral epileptiform activity and its use in conjunction with Ep could hardly be recommended. Methoxyflurane because of its slow onset of action is not useful for the induction of anaesthesia but offers good analgesia and muscle relaxation. The factor of importance when choosing a muscle relaxant is of importance where a rapid effect is required. Methoxyflurane because of its low dose related nephrotoxic effect (9) is seldom used as an inhalation anaesthetic but the dose administered in this case was well below the toxic dose. As a result of the necessitating immediate intervention because of asphyxia would have been associated with difficulties a planned caesarian section under general anaesthesia was considered to be the most desirable method of securing a maximum of security to both mother and

REFERENCES

- 1 Bjerkedal T & Bahna S L The course and outcome of pregnancy in women with epilepsy *Acta Obstet Gynecol Scand* 50 745 1973
- 2 Bonica J J Principles and Practice of Obstetric Analgesia and Anaesthesia Blackwell Scientific Publications Oxford 1969
- 3 Crawford J S The place of halothane in obstetrics *Br J Anaesth* 34 386 1967
- 4 Deffémunis Rospide H A & Vincent O Myasthenia yembarazo *Acta Neurol Lat Am* 20 75 1974
- 5 Evans D E N Anaesthesia and the epileptic patient *Anaesthesia* 30 34 1975
- 6 Foldes F F & McNall P G Myasthenia gravis A guide for anesthesiologists *Anesthesiology* 23 837 1967
- 7 Hay D M Myasthenia gravis and pregnancy *J Obstet Gynaecol Br Comm* 76 33 1969
- 8 Knight A H & Rhind H G Epilepsy and pregnancy A study of 153 pregnancies in 59 patients *Epilepsia* 16 99 1975
- 9 Mazze R J & Cousins M J Renal toxicity of anaesthetics with special reference to the nephrotoxicity of methoxyflurane anaesthesia *Cand Anaesth Soc J* 20 64 1973
- 10 McNall P G & Jafarova M R Management of myasthenia gravis in the obstetrical patient *Am J Obstet Gynecol* 97 518 1965
- 11 Miller H J Selman A H & Mickal A Myasthenia gravis of pregnancy at Charity Hospital Louisiana State M Soc 120 231 1968
- 12 Neigh J L Neuromuscular blockade *Surg Clin North Am* 55 837 1975
- 13 Perry A E & Livesley M Puerperal respiratory failure due to acute myasthenia gravis A case report *J Obstet Gynaecol Br Comm* 74 773 1967
- 14 Plauche W C Myasthenia gravis in pregnancy *Am J Obstet Gynecol* 88 404 1964
- 15 Vincent M Bustos R Rugga R Fonseca D Bértola M Effer S & Deffémunis H A Myasthenia gravis and pregnancy *Excerpta Med* 296 79 1973

Submitted for publication Febr 27 1977

P J Moberg
Department of Obstetrics and Gynecology
Karolinska sjukhuset
S-10401 Stockholm
Sweden

RUPTURE OF THE SPLEEN DURING DELIVERY

Susanne Christau and Joachim G Klebe

*From the Department of Obstetrics and Gynecology Rigshospitalet
University of Copenhagen Denmark*

Abstract A case of traumatic rupture of a normal spleen in a complicated delivery is presented. The etiology of splenic rupture and therapeutic aspects of this unusual occurrence are discussed. It is pointed out that a violent impression of the fetus might be dangerous for the mother.

Cases of rupture of the splenic artery, the spleen or even usually of an aneurysm of the splenic artery in association with child birth are very rare (1-15, 17). Saxtorph in 1803 first reported a case of ruptured spleen during pregnancy (12). The etiology of rupture of the spleen has been generally listed as being spontaneous due to toxemia or by trauma. The following case is presented as of interest primarily because of its unusual occurrence as one of the first traumatic ruptures in relation to a complicated delivery and because successful treatment adds a further maternal factor in a condition which has always carried a heavy mortality.

CASE HISTORY

A gravida aged 24. The pregnancy was uncomplicated until from 2 weeks past term. Labour began spontaneously 10 hours before she was admitted to a private clinic in the second stage 3 hours before. In the clinic delivery a vacuum-extractor and forceps was attempted but with little success and the patient was transferred to our department where the physical examination revealed the uterus to be soft and term sized but the left and right hypogastric areas were tender on palpation. A caesarean section was performed and she was delivered of a 4580 g healthy infant. Apgar score 7 after 1 min and 10 after 5 min. The recovery was uneventful until the 6th postoperative day. On the afternoon after straining at the stool she suddenly collapsed. The pulse was increased and the blood pressure were 50/40 mmHg. Immediate attention was given in resuscitation. Oxygen was administered by mask and transfusion commenced with plasma-expander (Maccel). The provisional diagnoses were made of embolism or hypovolaemic shock. The whole abdomen seemed tender there was a slight dyspnoea. The G did not show any sign of lung embolism. The pa-

tient's condition became worse and she was transferred to the emergency department. There the condition was complicated by cardiac arrest.

Laboratory studies disclosed the following values: Haemoglobin 2.0 mmol/l, base excess 30 mEq/l, standard bicarbonate 10.5 mmol/l.

The patient was oxygenated through an endotracheal tube and external heart massage was performed. The abdomen seemed then to be enlarged both flanks being dull to percussion. After a blood transfusion of 4 units unmatched blood and intravenous administration of 1 litre of plasma expander and 1.5 litres saline her general condition improved sufficiently that a laparotomy could be performed. The abdomen was opened by the caesarean section incision. The peritoneal cavity was found to contain fresh blood and clots. The uterus and tubes were found to be normal. On extending the incision up in the left curvature the blood was squirting from the region near the hilus of the spleen but the enormous quantity of free blood made examination difficult. The splenic vessels were clamped and splenectomy was performed. A 4 cm and a 1.5 cm laceration were noted on one pole of the surface of the spleen.

Besides the haematoma around the spleen some organized haematomas on the omentum at the curvature major at the stomach were found. The patient received 24 units blood, 3 units plasma-expander and a few litres of saline.

At the end of the operation the patient's condition had improved considerably. She made a remarkable uneventful recovery and was released on the 13th postoperative day.

The spleen measured 4x7x12 cm. The pathological report revealed no abnormalities in the spleen other than the lacerations.

DISCUSSION

Rupture of the splenic vessels or the spleen is a very rare complication of pregnancy and is as a rule rapidly fatal. The patient dying usually before operation can be performed. The mortality is high only 17 survivors of 61 cases of massive abdominal bleeding have been reported (4). Trauma is one of the most common causes of rupture of the spleen in non pregnant persons. The trauma may be external in the form of penetrating or non penetrating injury (16). The trauma may also be internal with such

causes as vomiting sneezing bending - coitus straining at the stool being implicated (2)

Rupture of the spleen in pregnancy is reported in 76 cases (6-12-13). It is interesting to note that in the large proportion of the published cases the bleeding is biphasic usually within hours of the trauma. In this patient the history spread over 3 days. In our case the traumatic attempt of the delivery together with the increased abdominal pressure and the elevated central venous pressure (3) due to the manual expression of the fetus before the admission may be responsible for the lesions of the spleen. The trauma at the failed vaginal delivery caused lacerations of the splenic parenchyma with or without rupture of the splenic capsule resulting in initial bleeding which is tamponated by blood clots. The delayed hemorrhage or biphasic rupture occurred by rejecting the blood clots or by secondary rupture of the capsule after straining at the stool.

Violent expression of the fetus therefore not only can reduce the intervillous blood flow but might also result in a dangerous condition for the mother.

Splenectomy is a treatment of choice for a ruptured spleen along with liberal blood transfusion.

REFERENCES

- 1 Abramovitch D R, Francis W & Helsby C R. Two cases of ruptured aneurysm of splanchnic arteries in pregnancy with comment on the lesser sac syndrome. *J Obstet Gynaecol Br Comm* 76: 1037 1969.
- 2 Donhauser I L & Locke H J. Traumatic rupture of the spleen. An analysis of sixty cases. *Arch Surg* 80: 1013 1960.
- 3 Klebe J G & Bay J. Måling af centralt venetryk under anæstesi. *Nord Med* 81: 12 1969.

- 4 MacFarlane J R & Thorbjarnarson B. Rupture of splenic artery aneurysm during pregnancy. *Am J Obstet Gynecol* 95: 1025 1966.
- 5 Ma Leod D C & Lond M S. Rupture of splenic artery. *Lancet* May III 974 1940.
- 6 McCammon III E. Rupture of the spleen during pregnancy. *J Fla Med Assoc* 58: 21 1971.
- 7 Pedowitz P & Perelli A. Aneurysms complicating pregnancy. *Am J Obstet Gynecol* 73: 770 1957.
- 8 Reed III F & Gupta R. Spontaneous rupture of the splenic artery in pregnancy. *Int J Gynaecol Obstet* 11: 29 1973.
- 9 Riva H L, Pickhardt W L & Breen J L. Rupture of splenic artery aneurysm in pregnancy. Report of a case. *Obstet Gynecol* 10: 569 1957.
- 10 Rogers W S, Morse I S & Sechinger D L. Postpartum rupture of a splenic artery aneurysm. Report of a case. *Obstet Gynecol* 24: 616 1964.
- 11 Rydberg H. Ruptur av lienans aneurysm vid graviditet. *Nord Med* 65: 787 1961.
- 12 Saxtorph M. *Gesammelte Schriften geburtshilflichen praktischen und physiologischen Inhalts*, p. 279. Copenhagen 1803.
- 13 Smith E H. Rupture of the spleen in pregnancy. *Natl Med Ass* 63: 781 1971.
- 14 Vassalotti S B & Schaller J A. Rupture of splenic artery aneurysm in pregnancy. *Obstet Gynecol* 30: 264 1967.
- 15 Schug J & Rankin R P. Rupture of a splenic artery aneurysm in pregnancy. *Obstet Gynecol* 26: 77 1965.
- 16 Wilcox H L. Nonpenetrating injuries of abdomen causing rupture of the spleen. *Arch Surg* 80: 88 1965.
- 17 Wylie I G. Spontaneous rupture of the splenic artery in early pregnancy. *Postgrad Med J* 4: 46 1966.

Submitted for publication Jan 24 1977

Joachim G Klebe
Kommunehospitalet
Dept. of Obstetrics and Gynecology
8000 Århus C
Denmark

MASSIVE ENLARGEMENT OF OCCLUDED TUBES AFTER POSTMENOPAUSAL TREATMENT WITH NATURAL ESTROGENS

Mats Hammar and Ulf Larsson Cohn

From the Department of Obstetrics and Gynecology, University of Linköping, Linköping, Sweden

We here report a probable side-effect of postmenopausal estrogen replacement that to the best of our knowledge has not been described previously.

CASE HISTORY

The patient was a primipara having had a normal delivery at the age of 37. She had no history of pelvic inflammatory

disease and the menopause had occurred by the age of 43. Four years later bilaterally occluded tubes were diagnosed at laparoscopy. One of the tubes was of normal size while the other was somewhat enlarged. The latter was emptied of 30 ml of clear fluid after which it totally collapsed.

When the patient was 56 years old she had still marked postmenopausal vasomotor symptoms. Pelvic examination showed no abnormalities and she was prescribed a combination of micronized oestradiol 4 mg+oestriol 2 mg to be taken cyclically with every 7th week free of medication.

All symptoms disappeared promptly and when she was examined 7 months later no pelvic abnormalities were noted. Eleven months later she was still symptom free but pelvic examination performed by the same gynaecologist as previously revealed a cystic mass.

At laparoscopy followed by laparotomy greatly enlarged hydrosalpinges were found (Fig. 1). They were excised and the postoperative course was uneventful. The tubes were 15 cm long with a maximal width of 5 cm. Both were filled with a clear fluid containing a few epithelial cells and some lymphocytes. The walls of the tubes were very thin and covered with a normal single layer epithelium.

DISCUSSION

During oestrogen administration to oophorectomized rabbits the tubal epithelium regains its secretory activity rapidly (2) and the number of regenerated secretory cells depends upon the dose administered (3). Clewe & Mastrianni (1) have reported that after castration the oviductal secretion of the rabbit fell rapidly but was restored after treatment with oestradiol.

In the Rhesus monkey the maximum production of tubal fluid usually occurs within the day of maximal vaginal cornification and it was considered likely that the secretion was induced by oestrogens (5). In the human female the oviductal epithelium shows its maximum secretory activity during the



Fig. 1. Photograph showing the enlarged tubes in situ.

follicular phase of the menstrual cycle (4). It would thus seem in the human female that endogenous oestrogen production stimulates tubal secretory activity.

It can therefore be suspected that in the case presented the fairly high doses of the two natural oestrogens estradiol and estron stimulated the production of the relatively large amounts of fluid which filled the occluded tubes. It would be interesting to know if equipotent doses of synthetic oestrogens could have a similar effect.

REFERENCES

- 1 Clewe T H & Mastroianu L Jr A method for continuous volumetric collection of oviduct secretions. *J Reprod Fert* 1: 146 1960
- 2 Flerc6 H Die Epithelien des Eileiters und ihre

hormonalen Reaktionen. *Z Mikroskopische Anat Forsch* 61: 99 1954

- 3 Frednsson B Proliferation of rabbit oviduct epithelium after estrogenic stimulation with reference to the relationship between ciliated and secretory cells. *Acta Morph Neerl Scand* 2: 193 1959
- 4 Greenwald G S Endocrinology of oviductal secretions. In *The Mammalian Oviduct* (ed F S E H J & R J Blandau) ■ 187 The University of Chicago Press Chicago and London 1969
- 5 Mastroianu L Jr Shah U & Abdul Karim R Prolonged volumetric collection of oviduct fluid in the rhesus monkey. *Fertil Steril* 12: 417 1961

Submitted for publication March 6 1977

Mats Hammar
Dept of Obstetrics and Gynecology
University Hospital
S 581 85 Linköping
Sweden

LETTER TO THE EDITOR

Dear Sir

Your recent letter to the Editor (*Acta Obstet Gynecol Scand* 55 469-470 1976) by Drs Belfrage and Raabe raises certain questions concerning the use of epidural anesthesia (e a) in obstetrics. Since the clinical problems may be approached differently we welcome this opportunity to discuss some main points which may be of general interest among obstetricians.

Drs Belfrage and Raabe emphasize that in their experience nearly every second primiparae has to be delivered instrumentally in order to terminate the second stage of labour within 1 hour. We have shown that it is possible to keep the frequency of instrumental deliveries almost at the normal level without exceeding our limit of 1 hour for the second stage (1).

Drs Belfrage and Raabe correctly assume that we have a high frequency of episiotomies—in fact 90% primiparae. This figure has been constant for years and has not been influenced by the introduction of e a. Fundal pressure has been applied in certain cases but of course is not a routine procedure. Fetal/neonatal welfare was evidenced by cardiotocography and high Apgar scores. The latter finding has been supported by the work of Matouskova et al (2).

Instrumental delivery in Scandinavia usually by vacuum extraction cannot be regarded as completely innocent for the fetus. In addition to the own risks of vacuum extraction we would like to draw attention to the fact that severe retinal hemorrhage is five times more frequent in neonates delivered by vacuum extraction as compared to spontaneous delivery (3). We therefore maintain our opinion that it is of some importance to keep the rate of instrumental deliveries at a low level.

Concerning the duration of bupivacaine there is disagreement between Drs Belfrage and Raabe

and ourselves. However the number of doses given during labour will differ with the various routines for administering local analgesics.

We do consider the occurrence of meconium stained amniotic fluid during labour and/or early cardiotocographic signs of intrauterine distress to be an indication for e a. As stated by Bonica (4) this makes immediate intervention possible without using general anesthesia. Moreover in our experience elimination of maternal stress is frequently beneficial for the fetal condition.

The fact that only 4 of our 19 cases of intrauterine distress were delivered by caesarean section for cephalopelvic disproportion supports this assumption. The 1 min Apgar scores were 9-10 in 15 cases and 6-8 in the remaining 4 cases.

Jan Martin Maltau and Harald T Andersen
Department of Obstetrics and Gynecology
Rikshospitalet
Oslo, Norway

REFERENCES

- 1 Maltau J M & Andersen H T. Continuous epidural anaesthesia with a low frequency of instrumental deliveries. *Acta Obstet Gynecol Scand* 54 401 1975.
- 2 Matouskova A, Dotton J, Forsman L & Victorin L. An improved method of epidural analgesia with reduced instrumental delivery rate. *Acta Obstet Gynecol Scand* 54 231 1975.
- 3 Egge K & Lyng E. Personal communication. To be published.
- 4 Bonica J J. In Principles and practice of obstetric analgesia and anesthesia. p 160. F A Davis Company Philadelphia 1977.

Submitted for publication May 9 1977

J M Maltau
Dept of Obstetrics and Gynecology
Rikshospitalet
Oslo
Norway

ANNOUNCEMENTS

An International Symposium on Cryptorchidism will be held in Stresa, Italy, between June 8-11 1978. Further information can be obtained from: Organizing Secretariat, Sirono Symposia, Via Primaticcio 158, 20127 Milano, Italy.

During the 6th European Congress of Perinatal Medicine held in Vienna, Austria, from August 30 to September 1 1978, the *Maternity Prize* of the European Society for Perinatal Medicine will be awarded for outstanding experimental, clinical and organizational achievements in the field of Perinatal Medicine. Persons who feel competent are kindly requested to nominate candidates not later than January 31 1978 to the President's address: Prof. Dr. O. Thalhammer, Univ. Kinderklinik, Währinger Gürtel 74 A, 1090 Wien, Austria.

First Congress of the International Society for the Study of Hypertension in Pregnancy 27-29 September 1978 in

Dublin, Ireland. Enquiries to: Prof. John Bonnar, Chairman, 1st Congress, Imap, I.M.A. House, 10 Fitzroy Place, Dublin 2.

The European Congress of Obstetric Anaesthesia and Analgesia is to be held at the National Exhibition Centre, Birmingham, England, in September 1979. The programme of the Congress is designed to be of interest to anaesthetists, midwives, obstetricians.

The Congress will last for four days and of the topics to be discussed will be:

Pain relief during labour, identification of and operative therapy for the foetus at risk, anaesthesia for operative delivery, neonatal resuscitation, intensive care of the seriously ill neonate and intensive care of the seriously ill parturient.

There will also be a full calendar of Social Events, a day for delegates, non-participating companions.

AN ELECTROIMMUNO ASSAY OF THE PREGNANCY SPECIFIC β_1 GLYCOPROTEIN (SP₁) IN NORMAL AND PATHOLOGICAL PREGNANCIES AND ITS CLINICAL VALUE COMPARED TO HUMAN CHORIONIC SOMATOMAMMOTROPIN (HCS)

S Sørensen

*From the Department of Clinical Chemistry and the Department of Obstetrics and Gynaecology
Frederiksberg Hospital Copenhagen Denmark*

Abstract A 95% reference interval for the pregnancy specific β_1 -glycoprotein (SP₁) was established on the basis of 799 samples from 254 normal pregnant women by electroimmuno-assay (rocket immunoelectrophoresis). A positive correlation was found between SP₁ and the human chorionic somato-mammotropin (HCS). The SP₁ concentration in maternal blood was halved about 30 hours after separation of the placenta at delivery. Thus emergency analyses would be of little value in detecting acute placental failure. 172 determinations of SP₁ in maternal sera were carried out on 56 pregnant women with various types of pathological pregnancies. To assess the clinical value of SP₁ a comparison with HCS (HPL) was made. It was concluded that SP₁ could possibly be a valuable parameter for monitoring and discovering pathological pregnancies. Larger investigations however must be performed to decide whether SP₁ determinations will provide more extensive information than other laboratory analyses presently used.

During human pregnancy the concentration of many of the normal serum proteins are changed. Increased amounts of α_1 -antitrypsin, α_2 -macroglobulin, transferrin and ceruloplasmin have been reported (5-11). Other proteins appear in the blood only during pregnancy. Bohn in 1972 isolated the pregnancy specific β_1 -glycoprotein (SP₁) from the human placenta and presumably it is synthesized there (2). SP₁ has also been identified by Lin et al. to be one of three pregnancy associated plasma proteins (PAPP-C) (10). In an earlier investigation Bohn detected SP₁ and three other pregnancy proteins in sera of pregnant women. He used the Ouchterlony gel diffusion technique with antisera obtained from rabbits immunized by injection of protein fractions from human placentas (1). Another pregnancy specific protein was identified as the human placental

lactogen (HPL) hormone also called human chorionic somato-mammotropin (HCS). The remaining two proteins SP₂ (identical with the steroid binding β globulin) and SP₃ (identical with the α_2 AP glycoprotein) are not specific for pregnancy. These two proteins can be detected in low concentrations in sera from normal women and men and in increased levels in patients suffering from various malignant and non malignant diseases in women using hormonal contraceptives and in men treated therapeutically with estrogens.

SP₁ has a β_1 -electrophoretic mobility and contains 28.1% carbohydrate (2). On the basis of electrophoresis in a sodium dodecylsulfate containing polyacrylamide gel the molecular weight has been determined to be $90\,000 \pm 5\,000$ (3). The biological function of SP₁ is unknown but SP₁ appears to have an affinity for estradiol, 17β -estradiol and cortisol (3).

The increasing production of SP₁ in placenta during pregnancy may provide a possibly useful placental parameter. The purpose of this study has been to establish a reference range and subsequently to assess the clinical application of SP₁ determinations carried out on serum samples from women with pathological pregnancies. In the groups where SP₁ estimations appeared to be of clinical value comparisons were made with the results of HCS analyses on the same samples since HCS is also produced in the placenta.

MATERIALS AND METHODS

Subjects

The study included 315 pregnant women all were admitted to the Department of

gynaecology

Table I The distribution of pregnant women (including age and parity) and samples according to diagnosis

Diagnosis	No of pregnant women	No of samples	Age (years)				
			≤14	15-19	20-24	25-29	30-34
Normal pregnancies	215	260		14	76	81	30
Induced abortion	39	39	1	1	8	18	8
Serial determinations in normal pregnancies	5	57					
Abruptio placenta	2	2			1	1	
Placenta previa	1	2				1	
Mild pre-eclampsia	9	31		1	3	4	1
Severe pre-eclampsia	3	11			2	1	
Chronic hypertensive disease	3	12			1	1	1
Neonatal asphyxia	7	17		1	3	1	1
Prematurity	12	36		1	5	3	3
Fetal death	11	76			2	5	3
Hydatidiform mole	3	5			3		
Twin pregnancy	5	26			5		
Total	315	528					

of 528 estimations of the pregnancy specific β_1 glycoprotein were made (Table I). To establish the reference interval, 260 serum samples from 215 pregnancies between the 17th and the 40th weeks of pregnancy were investigated. Results were included only for normal pregnancies for which the first day of the last menstrual period was known and which resulted in the delivery of a healthy infant with a birthweight $>2500 \pm 10$ days from the estimated term. Only cases in which the placenta was morphologically normal were included. The reference material was supplemented with 39 results from 39 pregnant women in whom induced abortions were performed. To provide an assessment of the course of development of β_1 concentration during the normal pregnancy, samples were drawn from five pregnant women at regular intervals during the pregnancy. The results were not used in determining the reference range.

Pathological pregnancies were divided into various subgroups (Table I). The definitions used for pre-eclampsia were those recommended by the American College of Obstetricians and Gynecologists Committee on Terminology (6). The criterion for neonatal asphyxia was an Apgar score of ≤ 5 one minute after delivery. If the birthweight was ≤ 2500 g the infant was regarded as premature (including true

premature born infants, genetically small full term infants and full term infants with intrauterine growth retardation).

Methods

Blood sampling. The samples were taken without anticoagulant and without special regard for status or precise time of sampling. When coagulation was complete the samples were centrifuged and the serum stored at -15°C until analyzed.

Antigen standard. A serum pool from pregnant women in the last trimester was collected in small portions stored at -15°C and used as an SP_1 standard. The pool was compared to a standard kindly supplied by Behringwerke AG Marburg, Lahn. No. 1093 containing 330 mg L-F $_2$. In 20 determinations the mean value was 168 mg/l, the S.D. was 4.6 mg/l and the coefficient of variation was 2.7%.

Antibody. Rabbit immunoglobulin against SP_1 , K 2416 was from Behringwerke Marburg, Lahn.

Assay buffer. 0.04 M sodium barbital buffer pH 8.6.

Crossed immunoelectrophoresis was carried out as described by Weeke (15). The standard was diluted 1+1 and 1 μl applied. The antibody concentration was 1% (v/v).

Electroimmuno assay (rocket immunoelectrophoresis). SP_1 was determined as described by Laurell (9) and Weeke (14). Glass plates ($205 \times 110 \times 1$ mm) and a 1 mm thick U shaped frame were used. To 1% (w/v) agarose (Lundström biologique Française) cooled to 40°C antibody was added to a final concentration of 1.25% (v/v). The solution was then poured into the mould. After gelation the non coated plate and the frame were removed and the wells punched out (diameter = 2.5 mm). The standard was diluted 1+1, 1+2, 1+5 and 1+10 with assay buffer (corresponding to 56, 28 and 15 mg/l). The samples were diluted 1+3. Only a few had to be used in 1+7 dilution. Samples from the eighth tenth weeks were used undiluted. 5 μl of standards and samples were applied with the current switched on 2-3.5 V/cm. Double determination

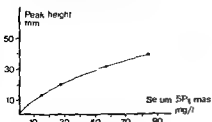


Fig. 1 A standard curve for SP_1 electroimmuno assay. Laboratory standard diluted to 56, 28 and 15 mg/l.

	Para II	Para III	Para \geq IV
8	12	6	
1			
1			
2	1		
3			
3			
4	1		
	1		
		1	
1			

samples and single determination of the standard dilutions were made. The electrophoresis was interrupted after 8–10 hours and the plate was covered with a wet filter paper and dried in hot air. Subsequently the plate was stained with Coomassie brilliant blue followed by destaining and drying. After electrophoresis of the plates with samples from the eighth–tenth weeks were placed for 30 min in a 0.1 mol/l NaCl solution followed by 30 min in distilled water. This procedure was necessary to prevent on precipitated proteins from extending the immunoprecipitates and making reading difficult. A standard curve for the plate in question was prepared with the SP concentration as abscissa and the peak height as ordinate. An illustration is given in Fig. 1. After the reading the SP concentration in the sample was calculated by multiplying the results by the dilution factor. The most sharply injected part of the curve corresponding to small SP₁ values was verified both for the standard from Behringwerke and for our own standard.

The HCS estimations were carried out routinely by a radioimmunoassay (8).

RESULTS

Methodological results

The specificity of the antibody was investigated by crossed immunoelectrophoresis. Only one precipitate can be observed (Fig. 2).

The precision of the method was assessed by interplate and intraplate variation. The coefficient of variation for double estimations on different plates of the same samples (mean value = 167 mg/l S.D. = 9.5 mg/l and $n=37$) was 5.8% and the coefficient of variation for double estimations on the same plate of one sample (mean value = 196 mg/l S.D. = 5.0 mg/l and $n=15$) was 2.7%.



Fig. 2 Crossed immunoelectrophoresis of 10 μ l antigen standard diluted 1+1. In the first dimension electrophoresis (10 V/cm for 1 hour) the anode is to the right. In the second dimension electrophoresis (7.5 V/cm for 20 hours) the anode is at the top. SP₁ antibody concentration was 1% (v/v).

The sensitivity expressed as the smallest concentration which could be measured was about 8 mg/l (the sample diluted 1+3). Using 5 μ l undiluted serum the sensitivity was about 2 mg/l.

The stability of SP₁ was investigated on three samples from three pregnant women in the last trimester. Sera were stored 8 days at -15°C, 4°C and room temperature. The results are given in Table II.

Clinical results

The reference interval. Since the number of SP₁ estimations during the course of pregnancy were rather small and since a Gaussian distribution of the estimations in the respective weeks could not be assumed, 2.5 and 97.5 percentiles have been used.

Table II The stability of SP₁ in three samples stored 8 days at -15°C, 4°C and room temperature

Sample	-15°C (mg/l)	4°C (mg/l)	Room temp (mg/l)
1	156	156	156
2	244	252	264
3	104	104	104

Table III The number of samples percentiles 2.5, 50 and 97.5 in the different weeks of normal pregnancy

Week of pregnancy	No of samples	Percentile		
		2.5 (mg/l)	50 (mg/l)	97.5 (mg/l)
8	19	3	5.5	8
10	20	3.5	6	14
12	12	14	16	20
14	17	18	20	31
16	14	21	28	36
18	15	20	28	51
20	17	25	34	47
22	17	28	32	52
24	15	37	46	59
26	18	44	60	87
28	15	47	72	96
30	20	64	76	125
32	20	79	116	152
34	20	76	142	191
36	20	80	183	293
38	20	100	199	292
40	20	92	138	225

(7) or rather a little bit higher and lower percentiles respectively. A percentile is shifted upwards depending on n (the number of estimations) so that the probability is 0.025 that less than 2.5% of the ranked values in a given week of gestation are below the designated percentile. Similarly a percentile is shifted downwards so that the probability is 0.025 that less than 2.5% of the ranked values are above the designated percentile (Table III). In Fig. 3 the calculated percentiles are smoothed graphically. SP_1 was detected as early as the eighth week. The concentration increased moderately in the first two trimesters but in the last trimester the increase was considerable. A decrease was seen only in the last week of the pregnancy. In the last months of the pregnancy great differences between individuals was observed. In the same figure serial SP_1 determinations on five pregnant women are shown. It can be seen that the level of SP_1 for one pregnant woman taken up around the 24th–26th week relative to those of the four other pregnant women was maintained during the rest of the pregnancy. The electroimmuno assay of serial samples from the pregnant woman with the highest levels of SP_1 is shown in Fig. 4.

Fig. 5 shows the correlation between SP_1 and HCS on 82 samples evenly distributed from the 24th–40th weeks of pregnancy. A positive correlation was found ($r = +0.64$).

The rate of SP_1 disappearance from the maternal blood after the separation of the placenta at delivery was studied on two women (Fig. 6). Samples were taken at the time of placental separation and at 12 hours intervals subsequently. The curves are non linear and do not permit calculation of half time but the SP_1 concentration is decreased about 45% 24 hours after separation of the placenta.

Abruptio placentae For the two pregnant women who developed abruptio placentae later in the pregnancy two SP_1 determinations were made in the 36th–38th weeks. The results were 80 mg/l and 188 mg/l which were below and within the reference interval. At term caesarean section was performed on both women because of abnormal fetal heart rates and vaginal hemorrhage. The Apgar score after one minute was ten in both cases.

Placenta previa Two SP_1 estimations were made on two samples from one pregnant woman in the 37th and 38th weeks. Both results were 138 mg/l which was within the reference interval.

Mild pre eclampsia SP_1 was estimated on samples from nine women with mild pre-eclampsia. There were no differences compared with normal pregnancies in the 36th, 38th and 40th weeks ($P > 0.05$ by Mann-Whitney's rank sum test) but except in the case of one woman the results were below the 50th percentile. An unchanged level was observed in one woman from the 34th to the 37th weeks. The placenta contained infarcts. All the infants were liveborn with a birthweight of 2500 g or more and an Apgar score of 8–10 after one minute.

Severe pre eclampsia 15 samples from five women in the 33rd–40th weeks with severe pre-eclampsia were studied. The range was from 88–

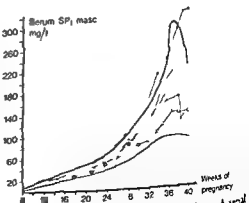


Fig. 3 The reference interval for SP_1 and serial estimations from five pregnant women with a normal pregnancy.

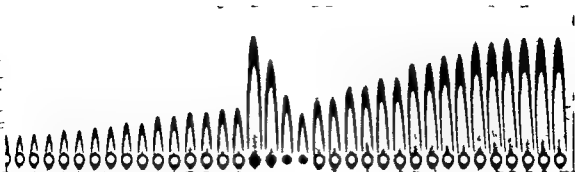


Fig 4 Electroimmuno assay with serial samples from the pregnant woman with highest level in Fig 2 ●=antigen standard dilutions (84, 56, 28 and 15 mg/l)

mg/l but apart from one estimation they were all within the reference interval although below the 0% percentile. The birthweights were 2200 g, 2500 g and 3700 g respectively. The Apgar scores after one minute were 9–10.

Chronic hypertensive disease Twelve serum samples from three pregnant women with hypertension were studied (Fig 7a). The range was from 23 to 100 mg/l. No. 2 in the figure showed a very small rise. The delivery took place in the 28th week. The infant's weight was 1010 g and the Apgar score after one minute was six. The next day the infant died of respiratory distress syndrome. Nos. 1 and 3 had nearly the same SP₁ concentration in the 35th–38th weeks and were in the lowest part of reference interval. The birthweights were 2100 g and 3150 g respectively. The Apgar scores after one minute were 9–10. HCS determinations on the same samples are presented in Fig 7b. For no. 2 an increasing concentration nearly up to the expected level in normal pregnancy was seen.

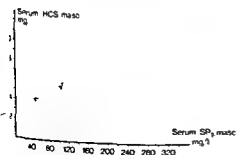


Fig 5 Correlation between SP₁ and HCS concentration in serum samples from pregnant women in the 34th–40th weeks ($y = 0.043x + 1.96$, $r = +0.64$, $P < 0.001$)

Neonatal asphyxia Analyses were carried out on 17 samples from seven women who gave birth to infants who showed symptoms of asphyxia after delivery (Fig 8a). Twelve results were scattered inside and five outside the reference interval. In four of five cases where several samples were available from the same woman during the pregnancy a rise in the SP₁ concentration to the level expected in a normal pregnancy was observed. The exception is no. 4 but nos. 3 and 7 whose infants had the lowest Apgar scores were below the reference interval.

Nearly all the corresponding HCS values (Fig 8b) fell in the reference interval including those for nos. 3 and 7.

Prematurity 36 samples from twelve women who delivered an infant weighing 2500 g or less were measured (Fig 9a). Information relating to the infant and the mother are presented in Table IV. Congenital malformations were in three infants (mothers nos. 2, 7 and 11). In two of these cases the

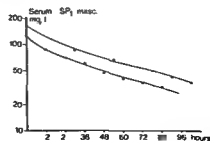


Fig 6 SP disappearance from maternal serum after the separation of the placenta. Two cases are shown.

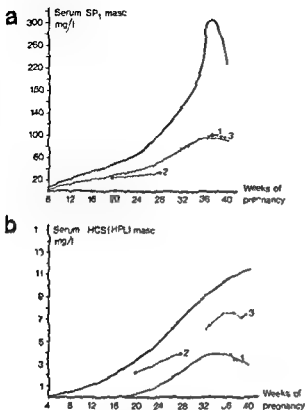


Fig 7 The serum concentration of SP₁ (a) and HCS (b) in three women with chronic hypertensive disease and the respective reference intervals

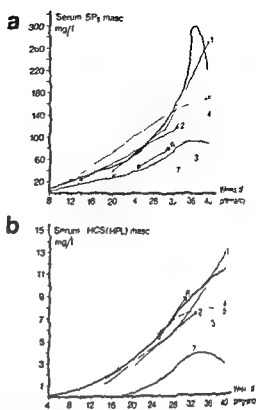


Fig 8 The serum concentration of SP₁ (a) and HCS (b) in seven pregnant women who gave birth to infants with neonatal asphyxia and the respective reference intervals

SP₁ level was above the reference interval (nos 2 and 11). In four women from whom samples were available after the 30th week and whose infants had birthweights of 2000 g or less and were without malformations the SP₁ results were below the re-

ference interval (nos 4, 5, 6 and 10). Two women with considerable placental infarcts had a pronounced SP₁ fall at the end of the pregnancy (nos 11 and 12).

With regard to the corresponding HCS values

Table IV Gestation age, birthweight, Apgar score and placental weight in women who delivered a premature infant

Pregnant woman (no.)	Week of pregnancy	Birth weight (g)	Apgar score 1 min after delivery	Placental weight (g)	Remarks
1	34-35	1 950	5	650	Danger of abortion in the 4th-5th months
2	28	1 475	3	570	Pulmonary tumor in the infant
3	40	2 300	7	—	
4	38-39	1 900	10	480	Infarcts and deg. change in the placenta
5	36-37	1 700	9	400	
6	36-37	2 000	10	650	
7	35	1 600	6	—	Down's syndrome, duodenal atresia
8	35	2 450	10	660	Large infarcts in the placenta
9	39	2 250	10	400	
10	32	1 510	—	—	
11	34	2 400	—	—	Congenital hydrocephalus
12	40	1 900	10	500	Infarcts in the placenta

Table V Fetal weight fetal changes placental weight and placental changes at fetal death

regnant woman (no)	Fetal weight (g)	Maceration	Placental weight (g)	Placental changes	Remarks
1	2 700	+	470	-	
2	274	+			
3	7 450	-	470	Deg. changes	Low urine estrogens
4	950	+	40	Deg. changes	
5	850	+	100	Necrosis	
6	840	+			An encirclage procedure was carried out in the 15th week
7					Spontaneous abortion
8	2 640	-	500	-	The mother had an impaired liver and renal function
9	3 500	-	560	-	
10	1 340	+	350	Deg. changes	Low urine estrogens the umbilical cord twisted tightly round the neck
11					Spontaneous abortion in the 15th week

Fig 9b) the values for only one woman were below the reference interval and showed a decreasing HCS concentration (no 5). A downward tendency was also observed in three other cases nos 4, 8 and 12).

Fetal death 26 SP₁ concentrations were measured in eleven women in whom pregnancy ended with the birth of a stillborn infant or in an abortion. Information relating to the fetus and the placenta are given in Table V and the SP₁ results are plotted in Fig 10a. The SP₁ concentrations for several of the women were above the reference interval at first but later on moved within the reference interval due to a small rise or fall of the SP₁ concentration (nos 1, 2 and 4). A high SP₁ value was observed in one woman who six weeks later was admitted to hospital with hepatitis and who delivered while suffering from this disorder (no 8). For three women the SP₁ values were below the reference range (nos 5, 7 and 11). A rising SP₁ concentration as expected in a normal pregnancy occurred in two cases (nos 3 and 10).

HCS determinations on the same samples (Fig 10b) showed a fall or lack of increase in concentration in three pregnant women (nos 1, 4 and 5) whereas three women showed a rise nearly as great as expected in relation to the reference range (nos 3 and 10).

Hydatidiform mole SP determinations on five samples from three women with hydatidiform moles were carried out. Only one SP value was within the reference interval while the others fell below this range. Serial measurements of one woman showed

decreasing concentrations. Exactly the same relationship was seen in HCS estimations.

Twin pregnancy SP₁ was measured on 26 samples from five women with twins. Results for three women were outside the reference interval (nos 2

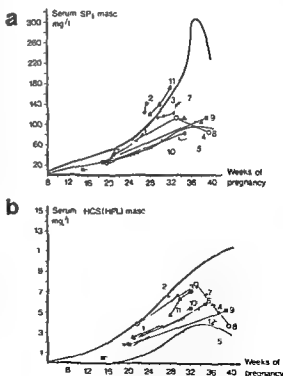


Fig 9 The serum concentration of SP (a) and HCS (b) in twelve pregnant women who delivered an infant weighing 2 500 g or less and the respective reference intervals

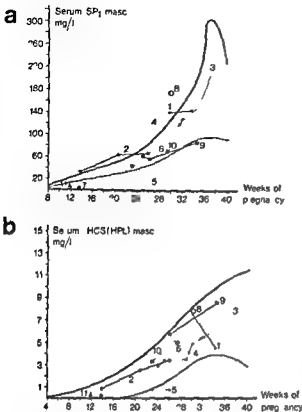


Fig 10 The serum concentration of SP₁ (a) and HCS (b) in eleven pregnant women in whom pregnancy terminated with a stillborn infant or an abortion and the respective reference intervals

3 and 4 in Fig 11a). In two cases the infant II died during a short time after the delivery (nos 3 and 4). Two monochorionic pregnancies had the highest and the lowest SP₁ levels (nos 1 and 2) respectively.

It can be seen in Fig 11b that the HCS results fell above or in the upper part of the reference interval.

DISCUSSION

There are only a limited number of reports about the pregnancy specific β_1 glycoprotein (SP₁) (1-4, 10, 12-13). SP₁ is presumed to be secreted by the placenta and occurs only in connection with pregnancy.

Storage experiments indicate SP₁ to be a thermostable protein which facilitates mailing of serum specimens and analyses in the laboratory.

The turnover of SP₁ in maternal blood after delivery designated as the half life, was determined by Bohn to be about 30-40 hours (3) and by Tatra et al to be about 34 hours (13). This estimate was confirmed by the current investigation where it

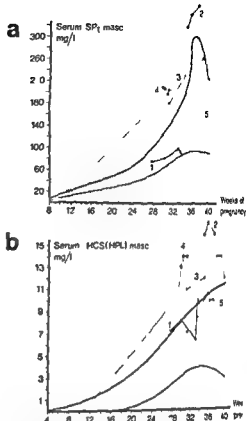


Fig 11 The serum concentration of SP₁ (a) and HCS (b) in five pregnant women with twins and the respective reference intervals

was observed that the SP₁ concentration was halved 24 hours after separation of the placenta. Therefore SP₁ is not suitable for detecting changes in placental function compared with HCS whose half life is much shorter (about 15-20 min). Thus emergency testing for SP₁ is of no value.

The rise of SP₁ in maternal serum during pregnancy is in agreement with Tatra et al (13) and in the current investigation the SP₁ level was significantly higher.

To assess the clinical value of SP₁ estimation it is logical to investigate the SP₁ serum levels in pregnancies terminating in fetal death. In seven women analysed with serial determinations two had an increase three had a smaller increase than expected and two had an increase as expected compared to the reference interval. An investigation of HCS concentrations yielded results essentially the same as for SP₁. In the two cases with normal SP₁ rise the 24 hours estimation was low. In one case the cause was that the umbilical cord was twisted tightly around the

one of three women in whom only one SP₁ estimation was performed the result was unexpectedly high. Six weeks later she was admitted to hospital with hepatitis. Laboratory tests showed that both liver and renal function were impaired. Perhaps the explanation of the high SP₁ value is that the liver and/or kidneys influenced the SP₁ half-life and the woman was subclinically affected at the moment of sampling. In a group of pregnant women with fetal death Tatra et al. found the serum SP₁ levels markedly reduced. Most values lay below the mean -2 SD level and in addition showed a downward trend in two cases with several samples (12).

As for neonatal asphyxia the SP₁ values for two women whose infants had the lowest Apgar scores were below the reference interval. The corresponding HCS values were in the lower part of the reference interval.

For the women who delivered an infant of 2000 g or less without congenital malformations the SP₁ results after the 30th week were below the reference interval. The corresponding HCS results were within the reference interval except in the case of one woman. Decreasing concentrations of both SP₁ and HCS were seen at the end of three pregnancies. For the five women who gave birth to twins the HCS results showed a tendency to be above or in the upper part of the reference interval than the SP₁ values.

Of other discoveries in this study it should be stressed that the SP₁ values in pre-eclampsia (except in the case of one woman) and in chronic hypertensive disease were in the lowest part of the reference interval. This is in accordance with Tatra et al. (12). They found the maternal SP₁ values in pregnant women with pre-eclampsia without essential fetal or placental impairment fell within the normal range but below the mean value of normal pregnancies. It is obvious that these pregnancies belong to the group of high risk pregnancies resulting from reduced placental function.

Generally information about changes in the placental function in a pregnant woman could be obtained by serial determinations and by observing percentile deviation later on in the pregnancy.

Conclusion SP₁ may possibly be a valuable parameter for monitoring and discovering pathological pregnancies. Larger investigations must be performed to decide whether SP₁ determinations will provide earlier and more extensive information than currently used hormone analyses.

ACKNOWLEDGEMENTS

The present work is an extract from an entry for a prize paper set by the University of Copenhagen, Denmark, and which was awarded the Gold Medal of the University.

The antiserum was kindly supplied by Behringwerke.

REFERENCES

- 1 Bohn H. Nachweis und Charakterisierung von Schwangerschaftsproteinen in der menschlichen Plazenta sowie ihre quantitative immunologische Bestimmung im Serum schwangerer Frauen. *Arch Gynaekol* 210: 440, 1971.
- 2 Bohn H. Isolierung und Charakterisierung des schwangerschafts-spezifischen β_2 -Glycoproteins. *Blut* 24: 297, 1972.
- 3 Bohn H. Untersuchungen über das schwangerschafts-spezifische β_2 -Glycoprotein (SP₁). *Arch Gynaekol* 216: 347, 1974.
- 4 Bohn H. Immunochemical determinations of human pregnancy proteins. *Arch Gynaekol* 217: 219, 1974.
- 5 Clarke H G M, Freeman T & Pryse Phillips W. Serum proteins in normal pregnancy and mild pre-eclampsia. *J Obstet Gynaecol Br Comm* 78: 105, 1971.
- 6 Greenhill J P & Friedmann E A. *Biological Principles and Modern Practice of Obstetrics*. W B Saunders Company, Philadelphia, 1974.
- 7 Herrera H S L. The precision of percentiles in establishing normal limits in medicine. *J Lab Clin Med* 52: 34, 1958.
- 8 Lebech P E & Borggaard B. Serum levels of human chorionic somatomammotropin (HCS) in normal and abnormal pregnancies. *Acta Endocrinol Suppl* 182: 35, 1974.
- 9 Laurell C H. Electromunological assay. *Scand J Clin Lab Invest* 29: Suppl 124: 21, 1972.
- 10 Lin T M, Halbert S P, Kiefer H & Spellacy W N. Three pregnancy associated human plasma proteins: Purification, monospecific antisera and immunological identification. *Int Arch Allergy* 47: 35, 1974.
- 11 Stimson W H. Studies on the changes in the concentration and total mass of individual serum proteins during late pregnancy. *Clin Biochem* 5: 3, 1972.
- 12 Tatra G, Breitenacker G & Gruber W. Serum concentration of pregnancy specific β_2 -glycoprotein (SP₁) in normal and pathologic pregnancies. *Arch Gynaekol* 217: 383, 1974.
- 13 Tatra G, Placheta P & Breitenacker G. Schwangerschafts-spezifisches β_2 -glykoprotein (SP₁). Klinische Aspekte. *Wien Klin Wschr* 87: 279, 1975.
- 14 Weeke B. Rocket immunoelectrophoresis. *Scand J Immunol* 2: Suppl 1: 37, 1973.
- 15 Weeke B. Crossed immunoelectrophoresis. *Scand J Immunol* 2: Suppl 1: 47, 1973.

Submitted for publication Aug. 11, 1976.

S. Sørensen
Bavnstedet 88
DK-3500 Værløse
Denmark

EXTREMELY LOW PLACENTAL LACTOGEN HORMONE (hPL) VALUES IN AN OTHERWISE UNEVENTFUL PREGNANCY PRECEDING DELIVERY OF A NORMAL BABY

Preben Gaede Dyre Trolle and Henning Pedersen

from the Departments of Obstetrics YA and G and the Department of Clinical Chemistry ML (Hormone Laboratories)
University of Hospital of Copenhagen Rigshospitalet and Herlev Hospital Copenhagen Denmark

Abstract A case of a normal pregnancy and delivery with extremely low placental lactogen hormone (hPL) values in maternal blood is presented. The low hPL-values were due to the fact that the placenta only produced about 1/25 the normal estimated output calculated on the basis of hPL-concentration in the intervillous spaces. The concentrations of progesterone, the placenta specific glycoprotein (SP₁) and total estradiol in serum were normal while prolactin and chorionic gonadotropin (hCG) were considerably elevated. Glucose levels were normal. On the ultrastructural level the actual placenta under study did not differ from a normal term placenta. In spite of the very low concentrations of hPL there was a good milk secretion and the mother was still breast feeding her baby 6 months after the delivery. Basal level of prolactin was thus time normal.

During the past five years the hormone human placental lactogen (hPL) has routinely been determined in the serum of 20 000 pregnant women. hPL is a specific placental hormone developed in the syncytiotrophoblast in increasing quantities as pregnancy proceeds. The hormone is to be found in the blood but not in the urine probably due to the fact that it is degraded in the maternal kidneys (2). Values above the reference interval are observed in cases of large placenta e.g. large fetus, multiple pregnancy, diabetes mellitus and Rhesus immunization. Furthermore, high values are found in women with renal failure due to insufficient degradation (4). Values below the reference interval are found in association with low values of urine and serum estradiol in cases of placental insufficiency or intrauterine fetal death or miscalculated length of pregnancy. Low values of hPL combined with normal values of estradiol are seen in an early stage of placental insufficiency (4).

During the five year period in which we have applied the hPL analysis routinely we have only once found extremely low hPL values in an otherwise

completely uneventful pregnancy. The case is hereby reported.

CASE REPORT

The patient was a 27 year-old primigravida whose last menstrual period was 14.2.1975 and who first felt fetal movements on 10.7.1975. Her expected date of delivery was 21.11.1975. Her pregnancy proceeded entirely uneventful apart from the reported hormonal values. The extremely low hPL values are shown in Fig. 1.

Due to an estimated fetal weight of 3 800 g and the fact that the patient was at term, medical induction of labour was performed on 21.11 and that same day a normal boy was delivered weighing 3 740 g, length 54 cm. Apgar scores were 10 after one and five minutes. The boy did not show any sign of abnormality. He was breast fed and mother and child were discharged from the hospital on the sixth day. The boy thrived and his weight at the time of discharge was 3 760 g. The placenta weighing 670 g was macroscopically and microscopically normal. Furthermore the placenta was examined by electron microscopy which will be referred to at a later stage.

The boy was examined at the age of 11 months and he was physically and mentally normal. He weighed 10.8 kg and measured 76.5 cm in length. He was still being breast fed and the mother stated that she produced ample amounts of milk in spite of the fact that she had started employment outside her home. At this examination she reported having menstruated (irregularly) on three occasions since the delivery.

MATERIALS AND METHODS

Maternal blood samples were drawn from an antecubital vein between 8 and 10 a.m. and analysed for hPL the same day. Afterwards the serum samples were stored at -20°C for subsequent analyses for estradiol, progesterone, prolactin, hCG and a placenta specific β glycoprotein (SP₁) (1).

Blood samples from aa and v umbilicals and the intervillous cavities were drawn by a special technique. Immediately after the delivery a piece of the umbilical cord about 15-20 cm was isolated with altogether four clamps (see Fig. 2). It is essential that this process is carried out as soon as possible in view of the fact that the aa umbilicals are emptied very quickly after delivery. By cutting the umbilical cord between the two sets of clamps (Fig. 2 (a) and (b)) the filled section of the umbilical cord

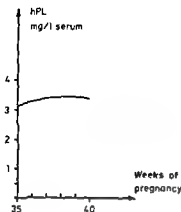


Fig 1 The extremely low hPL-values in the last part of pregnancy. The solid line is the lower limit of the 90% reference interval.

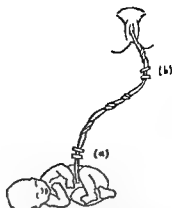


Fig 2 The special technique used for collecting blood from the aa and v umbilicales separately. The umbilical cord is cut between the clamps in (a) and (b).

was isolated. It was carefully dried with a piece of gauze (moistened with physiological saline) before collection of the blood samples. A hypodermic needle mounted on a syringe was then introduced into the v umbilicalis which was first emptied and subsequently into the two arteries which were now very distinct and readily emptied.

At the delivery of the placenta the membranes were carefully gathered round the placenta which was then placed in a large glass funnel. A few minutes later blood had collected between the cotyledons and a blood sample could readily be withdrawn with a syringe. This blood must be supposed to originate from the intervillous spaces of the placenta and is referred to as such in the following.

SP₁ and hPL in serum were determined by immunoelectrophoretic methods described in general by Nørgaard Pedersen & Gaede (3).

Serum prolactin, hCG and estronol were all measured by specific radioimmunoassays. Progesterone was measured by a competitive protein binding method.

Preparation for electron microscopy

Immediately after delivery of the placenta small tissue samples from different locations throughout the thickness of the chorionic plate were fixed in collidine buffered

glutaraldehyde for two hours and after a short buffer postfix in collidine buffered osmium tetroxide for another hour. After dehydration in graded concentrations of ethanol and propylene oxide the tissue was embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips 101 electron microscope.

RESULTS

Fig 1 shows hPL in serum during the last three weeks of pregnancy. The inserted line indicating the lower limit of the 90% reference interval for hPL in normal pregnancies (2).

Table I shows the concentrations of hCG, progesterone, SP₁, prolactin, glucose and total estronol in serum samples from the 38th, 39th and 40th weeks of pregnancy.

Table II shows the concentrations of serum hPL in the aa and v umbilicales and the intervillous spaces. The respective reference intervals here.

Table I The hormone concentrations in maternal serum during the last three weeks of pregnancy.

While the hPL values are extremely low, both hCG and prolactin show greatly elevated concentrations. Progesterone, SP₁ and total estronol are all within the normal range for this stage of pregnancy.

	hPL (mg/l)	hCG (i u/l)	Pro- gesterone (nmol/l)	SP ₁ (mg/l)	Total estronol (μmol/l)	Prolactin (μg/l)	Glucose (mmol)
38th week	0.4	119 700	530		0.65		4.6
39th week	0.7	103 000	760	160	0.69	ca 2 700	
40th week	0.6	109 500	810	195	0.80	ca 2 700	
11 months p.p.						65	

Normal range for hCG: 4 000–32 000 i u/l (38th–40th week)

Normal range for prolactin: 50–600 μg/l (38th–40th week) 80–150 μg/l (lactation period)

Table II Serum concentrations in aa and v umbilicales of hPL showing together with the concentration in the intervillous spaces an extremely low production of hPL in the actual placenta

	Serum hPL-concentration (mg/l)
maternal	0.7 (see Fig. 1)
aa and v umbilicales	<0.001 (0.01-0.03)
intervillous spaces	0.9 (25-37)

The concentration range shown in the brackets were calculated on the results of serum samples from five deliveries with normal hPL-concentrations in maternal serum.

Based on the basis of serum samples from five deliveries with normal maternal serum hPL concentrations.

Electron microscopy

The general ultrastructure of the term human placenta has been described in several reports with techniques of preservation changing through the years. Due to very elaborate ultrastructure of the cytotrophoblast particularly the syncytiotrophoblast, to the fact that the substructural details are highly dependent on the preservation technique, normal placenta were examined for comparison and referred to as the normal controls.

Syncytiotrophoblast In the placenta with extremely low production special attention was given to the endoplasmic reticulum of this cell. Both smooth and rough endoplasmic reticulum appeared approximately equal proportions when the actual placenta was compared to the normal ones. Usually there was a layer of smooth endoplasmic reticulum beneath the maternal surface of the cell (Fig. 3) and the interior of the cell areas appeared with tightly packed rough endoplasmic reticulum (Figs 3 and 4). The superficial smooth endoplasmic reticulum was of the flattened reticular type and only less well preserved cells did it show up as vesicular elements. Both types of the endoplasmic reticulum had a fine granular content. In the control placenta the smooth superficial endoplasmic reticulum occasionally appeared as fenestrated lamellae (Fig. 9) sometimes with a peculiar organization (Fig. 8) which has also been observed in human placenta. Such structures were not observed in the actual placenta associated with low hPL production.

In the actual placenta the mitochondria under investigation were found in the interior of the cell often in close relation to the rough endoplasmic reticulum (Figs 3 and 4). In number, location and substructure the mitochondria of the normal controls did not appear to differ significantly.

In both the normal controls and the actual syncytiotrophoblast cells the cytoplasm contained microfilaments, vesicular elements of different appearance, lipid droplets (Fig. 5) and superficially coated vesicles and rounded membrane bound bodies with a dense homogeneous content (Figs 3, 7, 8 and 9). Centrioles were seen (Fig. 3 inset) and often desmosomes were recognized within the cytoplasm apparently without any connection with the plasma membrane (Fig. 7).

Gold complexes were encountered throughout the cytoplasm but preferentially in the superficial cytoplasm and occasionally with dense material between its cisternae (Fig. 7). The maternal surface of the cell was characterized by the presence of numerous microvilli.

The nuclei had an irregular outline with peripheral clusters of heterochromatin in which light areas corresponded to the position of the nuclear pores (Fig. 5).

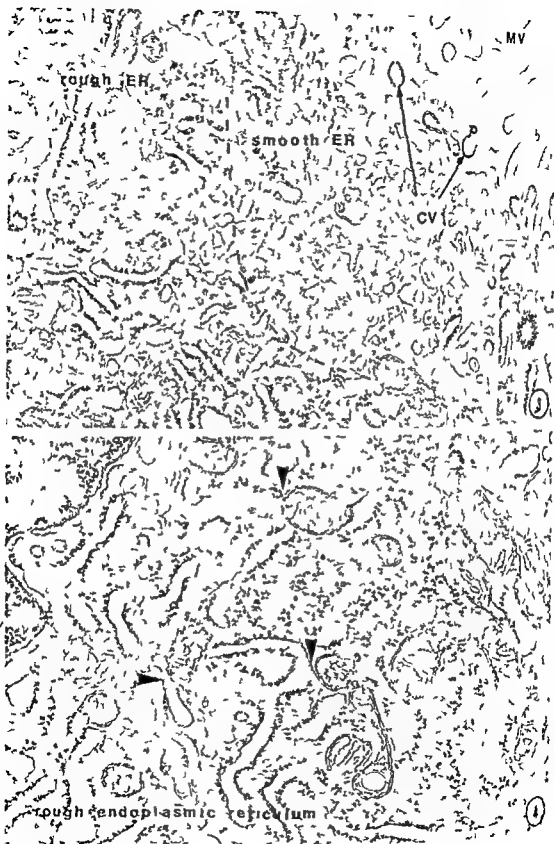
Cytotrophoblasts These cells were easily recognizable by their light cytoplasm with large mitochondria, scattered rough endoplasmic reticulum.

Fig. 3 From the actual placenta with extremely low hPL production. Beneath the maternal surface of the syncytiotrophoblast characterized by the microvilli (MV) and coated vesicles (CV) there is a layer of smooth endoplasmic reticulum. Deeper in the cell rough endoplasmic reticulum predominates. Inset: centriole.

Fig. 4 The mitochondria of such a cell (see Fig. 3) are closely related to the elements of the rough endoplasmic reticulum (arrow heads).

Fig. 5 In the cell type similar to the one shown in Fig. 4 the perinuclear cytoplasm is poorly provided with endoplasmic reticulum but dominated by bundles of microfilaments (MF) and smaller vesicular and membranous elements. Dispersed throughout the cell lipid droplets (LD) can be recognized. There is a normal distribution of heterochromatin in which light areas correspond to the nuclear pores (arrow-heads).

Fig. 6 The cytotrophoblast from the placenta with extremely low hPL concentration also has a normal substructure. The mitochondria are also in this cell type closely related to the profiles of rough endoplasmic reticulum (arrow heads). The cytoplasm is light with much fewer organelles than seen in syncytiotrophoblast cells. Microfilaments and microtubules are seen evenly dispersed in the cytoplasm (MF and MT).



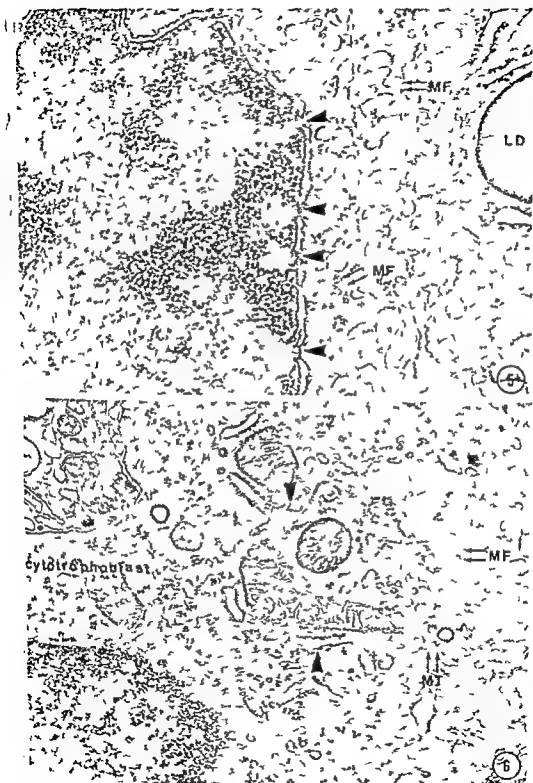




Fig 7 In normal placentae as well as in the actual one Golgi complexes were found mostly in the maternally oriented part of the cell and sometimes a dense material was interposed between the lamellae of this complex (arrow rows). Intracellular loose desmosomes were often observed (encircled).

Fig 8 In normal syncytiotrophoblast the endoplasmic reticulum was seen to be arranged in a peculiar structure (between arrow heads) a feature which was observed in the actual placenta under investigation.

Fig 9 Another structure that was only seen in actual placentae was fenestrated lamellae.

microtubules, microfilaments and glycogen (Fig. 6). Usually the rough endoplasmic reticulum was closely related to the mitochondria. In outline and substructure of the nuclei the normal controls did not seem to differ from the actual cells. Also in this cell type there seemed to be no significant difference between the normal placentae and the actual placenta.

DISCUSSION

Normal pregnancy and delivery with extremely low hPL-values during the last part of pregnancy have, to our knowledge, not previously been reported. As will appear from Table II, the low maternal hPL-values were due to the fact that the placenta only produced about 1/25 its normal output, estimated on the basis of the hPL concentration in the intervillous spaces.

Of the other hormone concentrations in the maternal blood, SP_1 , estradiol and progesterone were normal, while prolactin and also hCG were greatly increased. Also glucose levels were normal. A blood sample drawn from the mother 11 months after the delivery and while she was still breastfeeding showed a normal basal level of prolactin; at this time there had been three (irregular) menstrual like periods. It should be emphasized that throughout the period after delivery milk secretion has been adequate.

At the ultrastructural level the actual placenta did not differ from normal term placenta. The amount of cytoplasmic and nuclear elements, their intracellular location, their individual interrelationship and their substructure seemed to be normal. In the normal placentae, however, fenestrated lamellae and a whirled arrangement of smooth endoplasmic reticulum were recognized as unusual features which were not observed in the actual placenta. The functional significance of these modifications of the endoplasmic reticulum is presently unknown, but it seems unlikely that their unusual presence should be of any significance with respect to the production of the large amounts of hPL in normal pregnancies. Thus, from a morphological point of view, this functional defect in the actual placenta, viz. the almost complete lack of

hPL production, is not associated with significant cytological changes, at least with the present quality of cell preservation.

Perhaps one might not expect that a deficient production of a single protein hormone, even as pronounced as in this case, should be reflected in morphological changes in cells with such a multitude of functions as the trophoblast cells.

CONCLUSION

The purpose of the present study was to show that it was possible to carry through a normal pregnancy and delivery in spite of an extremely low hPL production in the placenta. The newborn child in question was entirely normal and showed no signs of physical or mental abnormalities at the age of 11 months. The mother had excellent milk secretion and was still breast feeding her baby 11 months after the delivery. The explanation might be found in the concentration of prolactin, which was considerably increased before the delivery but normal 11 months later at the post examination.

ACKNOWLEDGMENTS

We are grateful to Dr Claus Hagen and Dr Peter Schultz-Larsen for the prolactin and SP_1 analyses, respectively. The investigation was supported by the Danish Medical Research Council (Grant no. 512/2747).

REFERENCES

- 1 Bohn H. *Archiv Gynakol* 210: 440, 1971.
- 2 Gaede P. & Nørgaard Pedersen B. *Acta Endocrinol (Copenh)* 76: 369, 1974.
- 3 Nørgaard Pedersen B. & Gaede P. *Scand J Immunol Suppl* 119: 1973.
- 4 Trolle D., Bock J. E. & Gaede P. *Am J Obstet Gynecol* 126: 834, 1976.

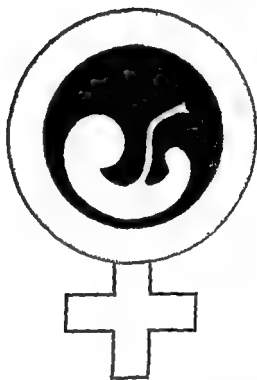
Submitted for publication June 8, 1977

Preben Gaede
Dept. of Clinical Chemistry ML
University Hospital of Copenhagen
Rigshospitalet
Copenhagen
Denmark

FOSTERÖVERVAKNING

Anencefali och Spina bifida
kan upptäckas redan på fosterstadiet

Serumanalys av alfafetoprotein (AFP)
i 16—19 graviditetsveckorna—
ett vardefullt hjälpmedel.



Phadebas AFP PRIST[®]

(för kvantitativ bestämning av AFP i serum och amnionvatska)

Svensk dokumentation *

- teknik
- klinik

*B Kjessler & S G O Johansson Alpha fetoprotein (AFP) in early pregnancy
Acta Obstet Gynecol Scand Suppl 69(1977)

For information kontakta
Pharmacia Norden AB Avd Diagnostika Box 159 751 04 UPPSALA 018/11 11 00



THE VALUE OF SERUM CYSTINE AMINOPEPTIDASE (CAP) HUMAN CHORIONIC SOMATO MAMMOTROPHIN (HCS) AND URINARY OESTROGEN ASSAYS FOR DETECTING INTRAUTERINE GROWTH RETARDATION

Gunnar Ryden

From the Department of Obstetrics and Gynecology University Hospital Linköping Sweden

Abstract The aim of the present study was to compare the usefulness of cystine aminopeptidase (CAP) and human chorionic somato-mammotrophin (HCS) estimations in maternal serum and maternal urinary oestrogen excretion in predicting intra uterine growth retardation. The material consists of 43 patients who subsequently gave birth to infants with a birth weight less than 2 S D from the mean according to the gestational age. The patients were controlled with simultaneous analyses of CAP, HCS and urinary oestrogen assays every week from the 36th week of pregnancy. The patients have been compared with another group in which infants with normal birth weight and without signs of fetal distress were delivered. Intrauterine growth retardation (IGR) was predicted by low oestrogen levels in 58%, low HCS levels in 42% and low CAP levels in 35%. The difference between the biochemical tests was not statistically significant. IGR infants who developed fetal distress were predicted by oestrogen assays in 71%, by HCS assays in 62% and by CAP assays in 8%. If urinary oestrogen assays were combined with HCS assays this combination predicted IGR significantly better than oestrogen assays alone. The combination HCS-CAP was as informative in this respect as urinary oestrogen assays alone. The results indicate that all methods tested are rather insensitive in predicting the total number of IGR infants. The capacity for predicting IGR infants with fetal distress is however rather high and for this purpose a combination of tests is preferable as no test fulfils the qualifications of being superior to the other. The combination oestrogens-HCS seems to be most suitable in this respect.

A review of mortality data from Sweden demonstrates a decreasing perinatal mortality during the last decade down to 1.3-1.4% (Fig. 1). The number of deaths during delivery and the neonatal period in recent years are mostly due to immaturity and malformations. Among the stillborn infants which are responsible for about 50% of the mortality there is an excess of small-for-date babies. About 90% of these infants weigh less than one S D from the mean according to the gestational age (5). A

further decrease in perinatal mortality might therefore be possible if better supervision of the pregnant women could be arranged during the last trimester in order to detect intrauterine growth retardation. Many different methods for evaluating the feto-placental unit during the last trimester have been published which indicate that no single test is perfect. Among the chemical methods in use measurements of urinary oestrol or total oestrogen excretion, estimation of human chorionic somato-mammotrophin (HCS) and cystine aminopeptidase (CAP) in maternal plasma seem to be the most widely used methods.

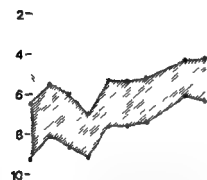
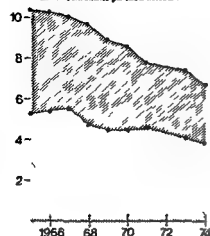
The aim of the present study is to compare the usefulness of all three chemical methods in predicting intrauterine growth retardation.

MATERIAL

The material has been selected from about 800 "at risk" pregnancies which were monitored by simultaneous analyses of CAP, HCS and urinary oestrogens usually with weekly intervals from the 36 week of pregnancy. The series consists of 43 patients who subsequently gave birth to infant with a birth weight less than 2 S D from the mean according to the gestational age. The patients were collected during the period 1 Jan 1973 to 31 Dec 1975. During that time the total number of deliveries was 6785 with a perinatal mortality of 1.15%. The small-for-date series has been divided into one group (A) including 22 patients without complications during delivery and another group (B) including 21 patients where fetal distress was observed at delivery (Apgar score less than 7 one minute after delivery or where the indication for operative delivery was fetal distress). Among the 43 patients studied in this way there was no perinatal death.

The "small-for-date" group has been compared with a series of 540 "at risk" pregnancies collected during the period 1 Jan 1973-31 Dec 1974 when the pregnancies were monitored in the same manner. These patients sub-

Number of still-born infants per 1000 newborn



Dead infants at 7 days after delivery per 1000 newborn

Fig 1 Perinatal mortality in Sweden 1965-1974. No figures are available for 1972 owing to change in account from the hospitals to the Medical Board. The bright area indicates infants with birth weight < 2500 g and the dark area birth weight \geq 2500 g.

sequently gave birth to single infants with normal birth weights and without signs of fetal distress during delivery. Patients who delivered infants with an Apgar score of less than 7 or where operative delivery was required for fetal distress (a total of 81 patients) have been excluded from the control group.

METHODS

The daily urinary output of total oestrogens was measured according to Hainsworth & Hall (7). The determinations were performed with a Technicon autoanalyzer. Cystine aminopeptidase (CAP) in serum was measured at 37°C with the method described by Peeters (8) using 1 cystine bis para nitroanilide as substrate. Human chorionic somato-mammotrophin (HCS) was measured according to Letchworth et al (8) whereby the Phadec kit from Pharmacia Diagnostics was used.

Low biochemical parameters have been defined as follows:

HCS < 4 mg/l serum from the 36 week of pregnancy
CAP < 80 nkat/l (4.8 U/l) serum from the 36 week of pregnancy

Urinary oestrol excretion

< 70 μ mol (20 mg)/24 hours during pregnancy weeks 36-37

< 84 μ mol (24 mg)/24 hours from 38 week of pregnancy

The statistical analyses performed were based on Fisher's exact test for two \times two tables (See Brownlee 1969).

RESULTS

The incidence of low biochemical parameters among women who delivered normal infants and small for date infants are presented in Table 1.

As demonstrated oestrogen assays seem to be somewhat superior to CAP or HCS assays in predicting growth retarded infants. In 58% of the women who delivered growth retarded infants low oestrogen values were found in comparison to 21% and 35% for HCS and CAP respectively. The figures obtained are rather low and indicate that biochemical methods are rather insensitive in predicting intrauterine growth retardation.

However, a comparison between small for date infants with uncomplicated deliveries and fetal distress clearly shows that the chemical assays and

Table 1 The incidence of low biochemical values in women who delivered growth retarded infants and normal infants respectively

Normal infants		Intrauterine growth retardation					
Normal infants		Totally A+B		Uncomplicated delivery (A)		Fetal distress (B)	
n	%	n	%	n	%	n	%
Number of patients	540	43		22		21	
Low CAP	29 5.4	15 35		5 23		10 48	
Low HCS	30 5.6	18 42		5 23		13 62	
Low oestrogens	47 8.7	25 58		10 46		15 71	

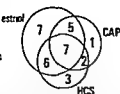


Fig. 2 The number of low biochemical tests with different combinations of tests in 43 mothers who delivered small-for-date infants

specially oestrogen assays to a large extent predict the complicated cases

A statistical analysis was performed in order to compare the usefulness of the methods in predicting intrauterine growth retardation. No significant difference was found in the total series (A+B) between the biochemical tests studied. Concerning the subgroups of uncomplicated delivery (A) and small-for-date infants with fetal distress (B) the numbers were too small to allow a statistical analysis.

In order to study if a combination of tests give more information than only one test, the incidence of low biochemical parameters have been calculated for the different combinations used. The number of low values when using different combinations of the biochemical tests are presented in Fig. 2 and Table II. A statistical analysis on the total series (A+B) was performed to evaluate whether oestrol combined with HCS or CAP is more informative than oestrogen assays alone. Oestrogen-HCS was significantly better than oestrogen alone ($P=0.003$), whereas when oestrogen-CAP was compared with oestrogens alone no such difference was found ($P=0.07$).

The results obtained with different combinations of tests are rather equal. The combination HCS-CAP seemed to be less informative than the

other combinations. A comparison between the combination oestrogen-HCS and HCS-CAP could however not demonstrate a significant difference in favour of oestrogen-HCS ($P=0.49$).

DISCUSSION AND CONCLUSION

Intrauterine fetal growth retardation secondary to placental insufficiency is responsible for a significant proportion of perinatal deaths in modern obstetrics (5). In order to detect placental insufficiency at an earlier stage of gestation different hormonal and enzyme assays have been suggested. In a recently published review Biggs (1) pointed out that opinions vary about the clinical significance of different assay procedures and oestrogen assays seem to be the yardstick against which many other tests are measured. In the present study urinary oestrogen assays were found to be the most useful for the prenatal diagnosis of growth retarded infants whereas HCS and CAP estimations were of similar value in this respect. The differences between the three methods were rather small and not statistically significant. Urinary oestrogen assays have however some limitations: the need for a 24-hour urinary sampling and the large day-to-day variation. HCS and CAP assays are easier to perform; the day-to-day variation is less and they are therefore more suitable for routine use. The recent introduction of quick radio-immunoassay procedures for free or total oestrol in serum and/or urine might however change the picture in this respect. Compared with radio-immunoassay procedures however the spectrophotometric method for CAP as such is considerably less expensive. If HCS and CAP were used together they predicted intrauterine growth retardation as effectively as urinary

Table II The incidence of low biochemical values with different combination of tests

	Intrauterine growth retardation							
	Normal infants		Totally A+B		Uncomplicated delivery (A)		Fetal distress (B)	
	n	%	n	%	n	%	n	%
Number of patients	540	43	22	21				
Low CAP or oestrogens	73	14	28	63	11	50	17	81
Low HCS or oestrogens	73	14	29	70	17	55	18	86
Low HCS or CAP	57	9.6	24	56	9	41	15	71
Low HCS, CAP or oestrogens	81	17	31	72	17	55	19	91

oestrogen assays. In the present study only patients examined from the 36th week of pregnancy were included in the study. It has been shown by Ryden (12) that CAP values generally decrease before oestrogen values in placental insufficiency and the same might be valid also for HCS. Studies performed earlier in gestation might therefore show some advantage for HCS and CAP assays in comparison with oestrogen assays for the early diagnosis of intrauterine growth retardation. Petrucco et al (10) who compared oestriol and CAP assays in predicting intrauterine growth retardation found CAP to be superior to urinary oestriol assays in this respect. The difference was only obvious between 28–35 weeks of gestation. CAP and HCS however only reflect the condition of the placenta. This implies that these methods cannot be a substitute for oestriol assays in monitoring at risk pregnancies but can be used as valuable tests for detecting growth retarded infants with an increased risk of fetal distress. No statistically significant difference was found between CAP and HCS assays in this respect. The at risk pregnancies detected in this way should be studied further with more sensitive indicators of fetal well being i.e. oestriol assays, CTG curves, oxytocin challenge tests.

It is possible that more information can be gained by serial assays of CAP in order to construct a curve and calculate the minimum and maximum allowable gradients for the change in enzyme activity. In that way Chapman et al (3) were able to predict small for date babies (less than the tenth percentile for gestational age) in 78%. They concluded that the gradient of CAP readings are more

important than the actual enzyme levels. From the clinical point of view a calculation of CAP gradients should increase the number of normal infants who were predicted as small for dates and it is therefore questionable if such calculations should offer any advantages. It should however be emphasized that rapidly increasing or decreasing levels of CAP might indicate an imminent intrauterine death of the fetus (11).

The incidence of low oestrogen values among small for date pregnancies in the present study (58%) is somewhat less than 72% obtained by Curzen & Varma (4). This is partly due to different criteria for low oestrogen excretion and partly to the definition of growth retardation. Thus Curzen & Varma found 21% low oestriol assays in normal birth weight infants compared to 9% in the present

study. They defined intrauterine growth retardation as a birth weight below the tenth percentile as compared with less than 2 SD from the mean in the present study. This latter fact also explains why Edwards et al (6) obtained low HCS levels in 7% of the growth retarded infants compared to 4% in the present study.

The high incidence (45%) of normal biochemical values in the group of growth retarded infants without fetal distress might indicate at least two different groups of growth retarded infants: one group, possibly genetic with normal placental function, normal biochemical tests and a low risk of complication during delivery and another group with placental insufficiency, low biochemical levels and a high risk of complications during delivery and the neonatal period. It can be emphasized that even if the biochemical tests are unreliable in the total number of growth retarded infants, all these methods are valuable in detecting those where fetal distress can be expected.

REFERENCES

- 1 Biggs J S G Progress in fetal assessment. *Gynecol* 43 277 1975
- 2 Brownlee K A Statistical Theory and Methodology in Science and Engineering III 163 Wiley New York 1965
- 3 Chapman L, Burrows Peakin R, Rege V P J, Silk E Serum cystine aminopeptidase and its small for dates baby in hypertensive pregnancy. *Br J Obstet Gynaecol* 83 238 1976
- 4 Curzen P & Varma M A comparison of serum stable alkaline phosphatase and urinary oestrogen excretion in the mother as placental function tests. *J Obstet Gynaecol Br Comm* 78 686 1971
- 5 Dahlén Nilsson I & Forsman L Perinatal mortalitet och födelsevikt. 5th Northern Congress of Perinatal Medicine Gothenburg III 1975
- 6 Edwards R P, Diver M J, Davis J C & High L J Plasma oestriol and human placental lactogen measurements in patients with high risk pregnancies. *Br J Obstet Gynaecol* 83 279 1976
- 7 Hansworth I H & Hall P E A simple automated method for the measurement of oestrogen in the urine of pregnant women. *Clin Chim Acta* 115 1971
- 8 Letchworth A T, Boardman R J, Bristol C, Landon J & Chard T A rapid semiautomated method for the measurement of human chorionic somatomammotrophin. The normal range in the third trimester and its relation to fetal weight. *J Obstet Gynaecol Br Comm* 78 542 1971
- 9 Peeters J A B M Automated determination of serum oxytocinase activity. *Clin Chem* 19 463 1973
- 10 Petrucco O M, Celier H & Fishall A Dupuis

of intrauterine fetal growth retardation by serial serum oxytocinase urinary oestrogen and serum heat stable alkaline phosphatase (HSAP) estimations in uncomplicated and hypertensive pregnancies. *J Obstet Gynaecol Br Comm* 80: 499, 1973

Ryden G. Cystine aminopeptidase in pregnancy. II. Its clinical application as an index of placental function. *Acta Obstet Gynecol Scand* 51: 379, 1972

Ryden G. Cystine aminopeptidase activity in pregnancy. III. A comparison between cystine

aminopeptidase activity in maternal blood and urinary oestrol excretion during pregnancy. *Acta Obstet Gynecol Scand* 53: 341, 1974

Submitted for publication Nov. 4, 1976

Gunnar Ryden
Department of Obstetrics and Gynecology
University Hospital
581 85 Linköping
Sweden

There is no substitute for quality



AB STILLE-WERNER

Box 43051 ☎ 100 72 STOCKHOLM SWEDEN

A/S Stille Werner C F Riche vej 103 DK 2000 København DANMARK

OY Stille AB Nervanderinkatu 5 D SF 00100 Helsinki 10 FINLAND

Stille AS Postboks 61 Leirdal Oslo 10 NORGE

Stille AG Postfach CH-8038 Zurich SCHWEIZ

Stille Werner (U.K.) Ltd 24 York Road Maidenhead Berkshire SL6 1SF ENGLAND

Stille GmbH Zulpicher Platz 7 D 5000 Köln 1 BRD

MANUFACTURING AND SALE OF surgical instruments operating
tables medical

STILLE

10 years
guarantee

ANTEPARTUM ADMINISTRATION OF TERBUTALINE AND THE INCIDENCE OF HYALINE MEMBRANE DISEASE IN PRETERM INFANTS

B Bergman and T Hedner

From the Department of Obstetrics and Gynaecology Centrallasarettet Malmö, Sweden

Abstract The incidence of hyaline membrane disease in preterm infants born between January 1975 and January 1976 was investigated in a retrospective study. Terbutaline, a β_2 -receptor stimulating drug, had been administered to the women in order to arrest premature labour. In spite of the treatment 24 preterm infants were delivered. The incidence of HMD in this group was 1/4. A group of 17 neonates whose mothers had received no treatment had an incidence of HMD of 5/17. Comparison between the two groups revealed a significantly lower incidence of HMD in the terbutaline treated group than in controls ($p < 0.05$). There were no significant differences in maternal age, gestational age, birth weight or Apgar score between the two groups. The lower incidence of HMD in the terbutaline treated group is suggested to reflect a rapidly induced release of pulmonary surfactants in the preterm infants. Evidence for a similar sequence of events has earlier been shown to occur in animals under standardized experimental conditions.

One of the major hazards of preterm infants is the development of idiopathic respiratory distress syndrome (IRDS) or hyaline membrane disease (HMD). A growing number of reports have been published concerning the prediction and prevention of HMD. In a controlled trial Liggins & Howie (10) showed that antepartum administration of glucocorticosteroids to the mothers lowered the incidence of respiratory distress syndrome of premature neonates. This observation was confirmed in an expanded study (6) and subsequently also by other investigators. It should, however, be noticed that Liggins & Howie in their trial actually used a combination of corticosteroids and drugs known to induce relaxation of the uterus, either sympathicomimetics or ethanol. Other drugs as thyroxine and heroin have also been reported to lower the incidence of respiratory distress syndrome (5, 15). Kero et al (7) did not observe any case of respiratory distress syndrome in 38 premature children whose moth-

ers have been treated with isoxsuprine during labour. A new category of drugs with selective β_2 -receptor stimulating effects as nitroderine, salbutamol and terbutaline has become available during recent years for suppression of uterine contractility (1, 8, 11). These drugs are extensively used in cases of threatened abortion, threatened premature labour and uterine hypercontractility during term labour. In a retrospective study Boog et al (3) found a reduced incidence of respiratory distress syndrome in premature infants following nitroderine administration to the mothers. The difference was only significant in infants weighing less than 2300 g compared with controls of equivalent weight. In this study we have investigated the effect of a β_2 -receptor stimulating drug, terbutaline, on the incidence of HMD in preterm infants.

PATIENT MATERIAL AND METHODS

Forty-one live born preterm infants delivered in the department between January 1975 and January 1976 were included in this study. The routine of the department in the management of preterm labour is administration of terbutaline orally or intravenously. Twenty-four infants were born before 36 completed weeks of gestation although the mothers had received terbutaline. Doses and administration routes appear in Table IV.

In some instances of preterm labour the mothers were not treated with terbutaline or any other β_2 -receptor stimulating drugs because of advanced labour at admittance to the hospital (7 infants), rupture of membranes at admittance to the hospital and delivery within 1 hour (6 infants), placenta praevia with hemorrhage (2 infants), signs of placental insufficiency (1 infant) and miscalculation of gestational age (1 infant). These 17 infants delivered by 15 mothers served as controls.

None of the mothers had received corticosteroids, isoxsuprine, thyroxine, heroin or any other drugs earlier reported to stimulate fetal lung maturation. premature rupture of membranes more

Table I Details of the terbutaline treated group

VD=Vaginal delivery CS=Caesarean section

Gestational age (weeks)	Maternal age (years)	Birth weight (g)	Terbutaline adm route	Apgar score	Sex	Delivery	HMD	Obstet comments	Pediatric comments
30	23	1 500	iv +o	9	M	VD	-	Cerclage	
30	27	1 650	iv	7	M	VD	-	Indomethacin 300 mg	
31	28	1 750	iv	7	M	VD	-	Indomethacin 100 mg	
31	19	1 910	iv +o	6	F	VD	-		
31	29	2 210	iv +o	8	M	VD	-	Vacuum extr	Immunosuppression
32	29	1 890	iv	3	M	VD	-	Indomethacin 300 mg	
33	27	1 590	o	10	F	VD	-	Multiple pregnancy	* and twin dead antepartum
33	19	1 940	iv +o	8	M	CS	-	Multiple pregnancy diabetes	
33	19	2 410	iv +o	7	M	CS	-	Multiple pregnancy Diabetes	
33	18	1 680	iv +o	7	M	VD	-		
33	19	1 650	iv +o	7	M	VD	-	Cerclage	
33	32	1 850	iv	7	M	CS	-	Ablatio plac	
33	34	2 260	iv	9	F	CS	HMD	Acute penapp	
34	29	2 070	iv +o	8	F	VD	-	Vac extr Cerclage	Immunosuppression
34	28	2 200	iv +o	8	M	VD	-	Cerclage	
35	26	2 420	iv	7	M	VD	-		
35	36	2 410	iv	4	M	VD	-		Listeriosis, dead after 3 days
36	23	2 435	iv +o	10	F	VD	-	Cerclage	
36	26	2 300	o	9	F	VD	-	Cerclage	Heart malformation
36	27	2 210	iv +o	10	F	CS	-	Multiple pregnancy Cerclage	Breast
36	27	1 820	iv +o	8	F	CS	-	Multiple pregnancy Cerclage	
36	23	1 850	iv +o	10	M	VD	-	Cerclage	
36	29	2 250	iv	10	M	VD	-	Toxemia	
36	26	2 440	iv +o	10	M	CS	-	Earlier hysterotomy	

fore delivery were excluded. Also excluded was one neonate in the terbutaline treated group who died within 2 hours post partum weighing 920 g and with a gestational age of 26 weeks. Unfortunately autopsy was not performed but no clinical signs of HMD were reported in this case. The preterm infants included in this study had a weight of 2 500 g or less.

All children were immediately taken to the neonatal intensive care unit observed given identical care and treated if complications developed. The diagnosis of HMD was based on the respiratory rate, grunting, chest retraction, increasing demand for oxygen, on radiological signs and on autopsy findings. The gestational age was based on menstrual history, uterine size, ultrasound scans, radiological examinations during pregnancy, birth weight, general appearance, body dimensions and neurological development at medical examination carried out immediately after birth (14). The Apgar score was recorded one minute after delivery and the weight of the infants were measured before or shortly after the transport to the neonatal intensive care unit.

Statistical analysis of the data included the χ^2 test and Student's *t* test.

RESULTS

Details and clinical data of the terbutaline treated group and the control group appear in Tables I and II. In the terbutaline group 24 infants were delivered by 22 mothers. One infant in this group showed typical signs of HMD. Of the 17 mothers delivered by 15 mothers in the control group was noted in 5 neonates. Comparison between the two groups revealed a significantly lower incidence of HMD ($p < 0.05$) in the terbutaline group. The mean gestational age in the terbutaline group was 33.6 ± 0.5 weeks (mean \pm S.E.M.) ranging from 30 and 36 weeks. The neonates of the control group had a gestational age between 31 and 36 weeks, mean 34.7 ± 0.4 weeks. The difference between the two groups was not significant. Compared with the control group a greater proportion of the infants in the terbutaline group had a gestational age of 33 weeks or less. In this subgroup one of 13 infants

Table II Details of the control group

V = vaginal delivery CS = Caesarian section

Infants (weeks)	Maternal age (years)	Birth weight (g)	Apgar score	Sex	Delivery	HMD	Obstet comments	Pediatr comments
19	1	470	3	M	VD	-		
24	1	570	7	M	VD	HMD	Multiple pregnancy	Dead after 27 hours
24	1	400	3	M	VD	HMD	Multiple pregnancy	Dead after 16 hours*
35	1	960	9	M	CS	HMD	Plac praevia	
36	1	890	9	M	VD	-		
37	2	180	10	F	VD	-		
25	2	040	8	F	CS	-	Plac praevia	
23	2	170	10	M	VD	HMD		
24	2	480	10	F	VD	-		
34	2	230	8	M	CS	HMD	Narrow pelvis	
37	2	290	10	F	VD	-		
39	2	470	10	F	VD	-		
30	2	340	10	M	VD	-		
24	2	120	10	F	VD	-		
37	2	280	10	M	VD	-	Multiple pregnancy	
32	1	540	8	F	VD	-	Multiple pregnancy	
40	2	340	10	M	CS	-	Diabetes	

*topsy showed heart malformation and pulmonary hyaline membranes

topsy showed intracranial hemorrhage and pulmonary hyaline membranes

terbutaline group and three of four infants in the control group developed HMD (Table III)

Comparing the terbutaline group with the control group there were no significant differences in maternal age (26.2 ± 1.0 years vs 26.8 ± 1.6 years) birth weight (2029.0 ± 62.5 g vs 2045.3 ± 85.6 g) or Apgar score (7.9 ± 0.4 vs 8.1 ± 0.8) (Table III)

even infants were delivered by caesarian section in the terbutaline treated group and of these infants developed HMD. This infant was delivered

in the 33rd week of gestation because of acute perianthecitis and the mother had received a total dose of 7.5 mg terbutaline intravenously during a period of 22 hours before delivery. In the control group 4 infants were delivered by c/s of which two developed HMD.

The total material includes three neonates delivered by diabetic mothers. These neonates were delivered by c/s none showed any signs of HMD.

Nine mothers in the terbutaline group had a cer-

Table III Comparison of the HMD frequency, gestational age, birth weight and Apgar score after one minute between the terbutaline treated group and the control group

HMD frequency	Terbutaline treated group 1/74	Control group 5/17	Statistical significance $p < 0.05$
Gestational age (weeks)	33.5 ± 0.4	34.7 ± 0.4	n.s.
Birth weight (g)			
total	2083 ± 63 n=24	2045 ± 86 n=17	n.s.
Weeks 36-34	2219 ± 67 n=11	2187 ± 70 n=13	n.s.
Weeks 33-31	1972 ± 81 n=11	1600 ± 1.5 n=4	n.s.
Weeks <31	1575 ± 75 n=7	-	-
Apgar score			
total	7.9 ± 0.4	8.0 ± 0.8	n.s.
Weeks 36-34	8.6 ± 0.6	8.9 ± 0.8	n.s.
Weeks 33-31	7.2 ± 0.5	5.5 ± 1.5	n.s.
Weeks <31	8.0 ± 1.0	-	-

*values are given as mean \pm S.E.M.

Table IV Dose and route of administration of terbutaline

Administration	Dose (mg)	Duration of treatment
Intravenously $n=8$	11 ± 3	26 hours ± 12
Orally $n=2$	236 ± 101	32 days ± 11
I.v. + orally $n=14$	18 ± 3	84 hours ± 13
	269 ± 66	19 days ± 3

Values are given as mean \pm S.E.M.

lage operation during pregnancy because of signs of cervical insufficiency earlier coisitation or a known twin pregnancy. According to the routine of the department all the patients with cervical cerclage were also treated with terbutaline.

One infant in the terbutaline group died three days post partum due to an intrauterine infection with *Listeria monocytogenes*. Clinical examination and autopsy showed no signs of HMD. Two of the infants in the control group delivered vaginally in the 32nd week of gestation of a twin pregnancy died both within 16 and 27 hours post partum respectively. Autopsy showed in both cases signs of typical HMD in one case also intracranial haemorrhage and in the other heart malformation.

Indomethacin was given to three of the mothers in the terbutaline group in an attempt to prevent premature delivery (Table I).

DISCUSSION

According to the classification of beta receptors in the subgroups of β_1 and β_2 (9) β_2 receptors are characterized to mediate uterine and bronchial relaxation and peripheral vasodilatation. Selective β_2 receptor stimulating drugs such as terbutaline, salbutamol and ritodrine have been found to effectively decrease both frequency and amplitude of uterine contractions (1, 8, 11).

Studies by Kero et al (7) and Boog et al (3) indicate that antepartum administration of beta stimulating drugs such as isoxsuprine and ritodrine decreases the incidence of respiratory distress syndrome in premature infants. In the study by Boog et al (3) the β_2 stimulating drug ritodrine was used. They found a reduced incidence of respiratory distress but the difference was only significant in infants weighing less than 2300 g. However this material was not grouped according

to gestational age which is an important factor in this context.

The present study indicates that terbutaline may be effective in preventing HMD as a result of reduction ($p < 0.05$) of the frequency of HMD occurring in preterm infants after antepartum administration. The terbutaline and control groups are comparable with regard to factors which are reported to influence the frequency of HMD such as gestational age, birthweight, maternal age, Apgar score, sex and maternal diabetes (17). It should also be noted that compared with the control group the terbutaline group contains more infants born at 34 and of lower gestational age which might indicate that the neonates of this group should have had a greater risk of developing HMD (these differences were not significant). However the two groups differ in this study in cases with advanced labour or rupture of membranes at admittance to the hospital given terbutaline.

Clinical studies have demonstrated a reduced incidence of respiratory distress in premature infants (6, 10) and an increased lecithine sphingomyelin ratio in amniotic fluid (4, 13) after corticosteroid administration early in the third trimester. In these studies the respiratory performance of the neonate was not observed until 24 h or more had passed following corticosteroid administration. However in many instances delivery takes place before any possible beneficial action of the corticosteroid has occurred.

In an experimental study (2) we have demonstrated increased pulmonary distensibility after terbutaline administration into fetal rabbits three hours before preterm delivery. This rapid effect of terbutaline on fetal rabbit lung maturation indicates that other mechanisms than stimulation of lecithine synthesis may be present. A possible mechanism might be an enhanced release of surfactant into the alveolar space as earlier suggested after isoxsuprine administration to fetal rabbits (1). However the possibility of a dilatation of the bronchioles or an alteration of the pulmonary blood flow in the fetuses should also be kept in mind.

The mechanisms behind the observed effect of terbutaline, the optimal dose and the timing of administration are currently being studied in our department.

For the time being it can be stated that antepartum terbutaline administration may be

useful for the prevention of HMD in preterm infants and that the effect of the use of beta stimulating drugs always should be kept in mind when evaluating results following corticosteroid administration.

ACKNOWLEDGEMENTS

The authors wish to thank Professor N. Wijkvist and Assistant Professor I. Kjellmer for reading and revising the manuscript.

REFERENCES

- Andersson H E, Bengtsson L, Ph Gustafson I & Ingemarsson I. The relaxing effect of terbutaline on the human uterus during term labor. *Am J Obstet Gynecol* 121/60 1975.
- Bergman H, Hedner T & Lundborg P. Effect of terbutaline on the pressure volume relationship in fetal rabbit lung. *Acta Obstet Gynecol Scand*. In press.
- Boog G, Ben Brahim M & Gandar R. Beta mimetic drugs and possible prevention of respiratory distress syndrome. *Br J Obstet Gynaecol* 82 85 1975.
- Ekelund L, Arvidsson G, Ohlander S & Åstedt B. Changes in amniotic fluid phospholipids at treatment with glucocorticoids to prevent respiratory distress syndrome. *Acta Obstet Gynecol Scand* 55 413 1976.
- Glass L, Rajagoweda H & Evans H. Absence of respiratory distress syndrome in premature infants of heroin addicted mothers. *Lancet* 2 685 1971.
- Howie R N & Liggins G C. Prevention of respiratory distress syndrome in premature infants by antepartum glucocorticoid treatment. In *Respiratory distress syndrome* (ed C A Villee, D B Villee & J Luckerman) p 369-380. Academic Press, New York and London 1973.
- Uro P, Hervoanen, T & Valmala, J. Prenatal and postnatal isoxuprine and respiratory distress syndrome. *Lancet* 2 198 1973.
- Landesman R, Wilson K H, Coutinho E M, Klima J M & Marcus R S. The relaxant action of nitodrine, a sympathicomimetic amine, on the uterus during term labor. *Am J Obstet Gynecol* 110 111 1971.
- Lands A M, Ludena F P & Buzzo H J. Differentiation of receptors sensitive to isoproterenol. *Life Sci* 6 2241 1967.
- Liggins G C & Howie R N. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics* 50 515 1972.
- Liggins G C & Waughan J S. Intravenous infusion of salbutamol in the management of premature labour. *J Obstet Gynecol Br Comm* 80 29 1973.
- Robert M F, Neff R K, Hubbell J P, Taeusch H W & Avery M E. Association between maternal diabetes and respiratory distress syndrome in the newborn. *N Engl J Med* 294 357 1976.
- Spellacy W N, Buhi W C, Riggall F C & Holsinger K L. Human amniotic lecithin/sphingomyelin ratio changes with estrogen or glucocorticoid treatment. *Am J Obstet Gynecol* 115 216 1973.
- Usher R, McLean F & Scott R. Judgement of fetal age. II. Clinical significance of gestational age and an objective method for its assessment. *Pediatr Clin North Am* 13 835 1966.
- Wu B, Kikkawa Y, Orzalesi M M, Motoyama H, K. Kaubara M, Zigas C J & Cook C D. Accelerated maturation of fetal rabbit lungs by thyroxine. *The Physiologist* 14 253 1971.
- Wyszogrodski I, Taeusch H W & Avery M E. Isoxuprine induced alterations of pulmonary pressure volume relationships in premature rabbits. *Am J Obstet Gynecol* 119 1107 1974.

Submitted for publication March 10 1977

Börje Bergman
Dept of Obstetrics and Gynecology
Sahlgrenska Hospital
S-413 45 Gothenburg
Sweden.

MIDTRIMESTER INTRA AMNIOTIC ADMINISTRATION OF PROSTAGLANDIN $F_{2\alpha}$ IN COMBINATION WITH AN HYPEROSMOLAR UREA SOLUTION EFFECT UPON PLASMA LEVELS OF ESTRADIOL PROGESTERONE AND HUMAN PLACENTAL LACTOGEN (HPL)

Geoffrey Sher and Maurice Katz

From the Gynecological Endocrine Service Department of Obstetrics and Gynecology University of Cape Town/Groote Schuur Hospital Cape Town South Africa

Abstract A study was undertaken in order to investigate clinical observation that patients who underwent first-trimester abortion using intra amniotic $PG F_{2\alpha}$ in combination with hyperosmolar urea always aborted a live fetus. Ten Caucasian primigravidae aged between 18 and 22 years and whose pregnancies ranged between 14 and 23 weeks in duration were studied. The patients were randomly divided into two equal groups. The one group received urea and $PG F_{2\alpha}$ intra amniotically whereas the other received $PG F_{2\alpha}$ alone. Blood was drawn for measurement of plasma estradiol, progesterone and human placental lactogen (HPL) prior to injection of the abortifacients and at regular intervals thereafter for a period of 120 h. The five patients who received the combination treatment (urea + $PG F_{2\alpha}$) showed a rapid decline in the plasma concentrations of these hormones and induction of abortion was followed by fetal death within 35 min in all cases. In contrast the five patients who received intra amniotic $PG F_{2\alpha}$ alone did not (with a single exception) demonstrate this rapid decline in the plasma concentrations of the placental hormones measured. Also in the same single exception these fetuses although born were alive two hours after inducing abortion.

Intra amniotic administration of prostaglandin in combination with hyperosmolar urea has been found to be an effective method of inducing first-trimester abortion (2-4, 6). The advantages that this method has over the use of intra amniotic prostaglandin $F_{2\alpha}$ alone outweigh the disadvantages. The hematologic and metabolic changes associated with the intra amniotic administration of prostaglandin $F_{2\alpha}$ in combination with urea are insignificant and are no more severe than when prostaglandin $F_{2\alpha}$ is injected alone (2, 3). Moreover, with the possible exception of myometrial necrosis, the complications associated with the intra amniotic administration of prostaglandin $F_{2\alpha}$ in combination with urea do not appear to be more severe than when prostaglandin is administered alone via this route. It should

be borne in mind that myometrial necrosis results from the intra myometrial injection of the abortifacients and that administration of the agents into the amniotic cavity via a correctly placed catheter will prevent the occurrence of this complication.

The advantages of using the combination regime (urea + prostaglandin) rather than prostaglandin alone are as follows:

(a) a single injection will achieve the desired result (2, 3, 6) and

(b) the fetus is always aborted dead. This contrast with the occurrence at times of live abortuses following intra amniotic administration of prostaglandin alone (6).

In our experience the incidence of gastrointestinal side-effects is not increased by using the combination regime (6).

This study was designed to measure and compare a few endocrine parameters of placental function in two matched groups of randomly selected patients. The one group received prostaglandin $F_{2\alpha}$ alone by intra amniotic injection while the other received prostaglandin $F_{2\alpha}$ in combination with urea via the same route. The exact time of fetal death was determined by the disappearance of heart sounds (when this occurred within 2 hours of the first intra amniotic injection).

PATIENTS AND METHODS

Ten Caucasian primigravidae aged between 16 and 22 years whose pregnancies ranged between 14 and 23 weeks gestation were selected for this study. The patients were characterized by the absence of organic disease and by the fact that without exception the indications for termination of pregnancy were psychological disturbance and/or psychiatric disease. Ultrasonic investigation confirmed the clinical observation that uterine size appropriate for gestational age in each case.

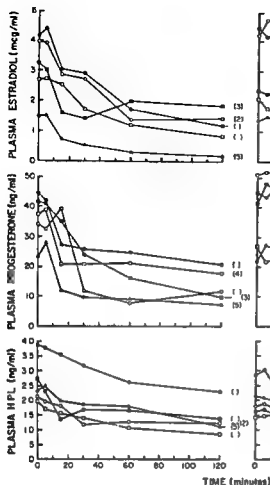


Figure 1

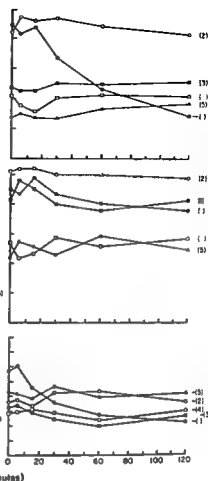


Figure 2

The patients were randomly divided into two groups of five each. Group A received 30 mg of prostaglandin F_{2α} in combination with 100 cc of a 60% sterile urea solution by intra amniotic injection and Group B received 30 mg of prostaglandin F_{2α} alone via the same route.

Blood was drawn immediately prior to the initial injection of the abortifacients and then at 5 min, 15 min, 30 min, 60 min, and 120 min following the procedure. The specimens of blood were assayed for plasma estradiol, progesterone, and human placental lactogen (HPL) concentrations.

Fetal heart sounds were recorded from the outset with a Doppler apparatus.

RESULTS

Graphic illustrations of serial plasma estradiol, progesterone, and HPL levels are compared in Groups A and B in Figs 1 and 2.

It will be noted from Figs 1 and 2 that in five cases where urea was administered in combination with prostaglandin F_{2α} via the intra amniotic route, a steady decline in the plasma concentrations of the

measured hormones occurred. A striking difference is shown in the group of patients who received prostaglandin F_{2α} alone. Four of the five patients in Group B showed no significant change in plasma estradiol, progesterone, or HPL levels.

In all five cases constituting Group A, the fetal heart became inaudible by Doppler within 35 min of injecting the prostaglandin and urea (range 19-60 min). In only one patient in Group B (patient 2) did the fetus die within two hours of the intra amniotic injection of prostaglandin F_{2α} alone; in this case fetal demise occurred within 3 min of injecting the prostaglandin.

DISCUSSION

Numerous publications have endorsed the use of prostaglandin when administered in combination with hyperosmolar urea. When administered alone, prostaglandin via the intra amniotic route potentiates prostaglandin-induced myometrial activity (3-6). Several studies have shown that

Fig 1 Serial plasma estradiol, progesterone, and HPL levels of 5 patients in Group A who received prostaglandin F_{2α} and hyperosmolar urea by intra amniotic injection. Patient (1)=23 weeks, patient (2)=16 weeks, patient (3)=16 weeks, patient (4)=15 weeks, patient (5)=14 weeks.

Fig 2 Serial plasma estradiol, progesterone, and HPL levels of 5 patients in Group B who received prostaglandin F_{2α} alone by intra amniotic injection. Patient (1)=19 weeks, patient (2)=18 weeks, patient (3)=14 weeks, patient (4)=14 weeks, patient (5)=16 weeks.

out three hours of the intra amniotic administration of prostaglandin $F_{2\alpha}$ alone the peripheral plasma progesterone concentration starts to decline.

5) The same studies also suggest that the rate of decline correlates well with the installation abortion interval.

The evidence produced by our study corroborates the findings of Craft et al. that the combination of urea and prostaglandin produces a rapid and profound decline in placental function resulting in rapid fetal demise and that the rate and degree of decline of placental endocrine function is more rapid and more constant than that which occurs following the intra amniotic administration of prostaglandin alone. It is concluded that a single intra amniotic injection of urea in combination with prostaglandin $F_{2\alpha}$ ensures the rapid midtrimester abortion of a dead fetus (2-3-6) and as such represents an excellent alternative to the intra amniotic installation of prostaglandin $F_{2\alpha}$ alone.

REFERENCES

1. Aleem F A, Schulman H, Saldana L R & Hin Cheung Hung. The effect of prostaglandin $F_{2\alpha}$ on the

- placental progesterone level in midtrimester abortion. *Am J Obstet Gynecol* 123: 202, 1975.
2. Burkman M T, Atienza M F, King T M & Burnett L S. Intra amniotic urea and prostaglandin $F_{2\alpha}$ for midtrimester abortion. A modified regimen. *Am J Obstet Gynecol* 126: 379, 1976.
3. Craft I L, Walker M & Youssefnejadian F. Intra amniotic prostaglandin $F_{2\alpha}$ and urea for abortion. *Prostaglandins* 5: 397, 1974.
4. King T M, Atienza M F, Burkman M T, Burnett L S & Bell W R. The synergistic activity of amniotic prostaglandin $F_{2\alpha}$ and urea in the midtrimester elective abortion. *Am J Obstet Gynecol* 120: 704, 1974.
5. Saldana L, Schulman H, Yang W H, Cunningham M & Randolph U. Midtrimester abortion by prostaglandin impact. *Am J Obstet Gynecol* 44: 579, 1974.
6. Sher G. Therapeutic midtrimester abortion by the intra uterine administration of prostaglandins. *South Afr Med J (Suppl. South Afr J Obstet Gynecol)* 39: 1173, 1976.

Submitted for publication July 4, 1977

Geoffrey Sher
Dept. of Obstetrics and Gynecology
University of North Carolina School of Medicine
Chapel Hill N.C. 27514
USA

INTRAUTERINE INJECTION OF VITAMIN K BEFORE THE DELIVERY DURING ANTICOAGULANT THERAPY OF THE MOTHER

Jørgen Falck Larsen Bo Jacobsen Hans Henrik Holm
Jan Fog Pedersen and Margit Manton

*From the Department of Obstetrics and Gynaecology the Coagulation Laboratory
and the Ultrasonic Laboratory Gentofte and Herlev University Hospitals
Copenhagen Denmark*

Abstract The coagulation factors PP^{III} (Factors II+IV) and factor X were determined in infants born to mothers receiving anticoagulants. The factors were very low when the mothers did not receive vitamin K before the delivery. No significant improvement in the factors was observed after an intravenous or intraamniotic injection of vitamin K two to four days before the delivery. Vitamin K was injected intramuscularly into four mothers *in utero* using an ultrasonic multitransducer. Three of these infants had normal coagulation factors at birth. In the fourth case the factor X was normal and factor IX was significantly better than in the infants of other groups. The injection had no harmful effects to infants.

Thrombo-embolic disease is a serious complication in pregnancy. The incidence given by various authors varies from 0.018 to 0.29 per 100 deliveries (1). Turnbull et al. (17) stressed that thromboembolism is second only to abortion as a cause of maternal death. These authors reported that during six years 1961-1966 230 women died of thromboembolism associated with pregnancy in England and Wales, a fatality once in every 25000 pregnancies. Two of the three maternal deaths which occurred in Denmark during 1972 were caused by thrombo-embolic disease (3).

Although postnatal maternal death from embolism has tended to decrease, the incidence of perinatal embolism seems to rise (15). Therefore, an effective treatment for deep vein thrombosis is important to avoid the development of pulmonary embolism.

Before the use of anticoagulants, thromboembolism often ended in maternal death. Villa-Santa (8) collected 163 case histories from the literature with a maternal death rate of 12.8%. Yahr et al. (19) introduced the use of anticoagulants during preg-

nancy. Since then a number of publications have confirmed that maternal death can be reduced with anticoagulant therapy (1-18).

However, the anticoagulant therapy exposes the mother as well as the foetus to the hazard of haemorrhage. A number of authors reported maternal complications such as postpartum haemorrhage and haematoma in the episiotomy (4-16). As these maternal complications are negligible compared with the risks of thrombo-embolic disease, the major problem is foetal bleeding. In a comprehensive review Villa-Santa (18) found 92 cases of perinatal foetal death during anticoagulant therapy or a perinatal loss of 11.4%. The anticoagulant therapy also increases the risk of brain damage in surviving infants with a diminished clotting status at birth (2).

As the use of streptokinase is contraindicated in pregnancy (15), the medical treatment of deep thrombosis leaves a choice between anti-vitamin K agents such as the coumarins and indanediones and heparin. The dosage of coumarins and indanediones is easy to control by an experienced doctor, but the compounds pass the placental barrier. They are competing with vitamin K and effect the factors II, VII, IX and X in the foetal blood.

Severe foetal damage or even foetal death has been reported in several cases with the use of the anti-vitamin K drugs. Especially the use of slow acting phenprocoumon (Marcomar®) seems to expose the foetus to serious risks when controlled with long intervals (8, 11).

Heparin has a molecular weight of 16000 and it does not pass the placental barrier. On the other hand, the heparin treatment is difficult to maintain for a long period. Heparin is only effective by injection intravenously or subcutaneously, and the control of the dosage is very difficult (5).

The anticoagulant therapy may be designed as a combination of the two methods using an oral anticoagulant until 37 weeks gestation followed by heparin until after delivery (6). This allows the foetus to eliminate the anti vitamin K drugs before delivery reducing the perinatal risk of haemorrhage.

This study was undertaken to evaluate the different methods and to improve the methods by injecting vitamin K into the foetus shortly before the delivery.

MATERIAL AND METHODS

The series consisted of eleven cases in which anticoagulant therapy was initiated before or during pregnancy. The indications for anticoagulant therapy were thromboembolic disease or—in one case—a Key-Shiley prosthesis. The women received treatment with phenindione by mouth until one or two weeks before the expected time of delivery. Then the anti vitamin K treatment was replaced by intravenous heparin. The phenindione treatment was controlled by frequent estimations of the Owen PP% (Factors II+VII) while the factors IX and X were screened in longer intervals.

After the termination of anti vitamin K treatment and the initiation of heparin injections different procedures were evaluated.

Group I In the first part of this study it was decided to perform a caesarean section in two cases assuming that this mode of delivery would be less traumatic for the foetus than a vaginal delivery. These mothers did not receive vitamin K before the delivery.

Group II Three mothers were given large doses of vitamin K (10–30 mg) intravenously between two and four days before the delivery. As vitamin K crosses the placental barrier it was expected to correct the coagulation status.

Group III In two cases the vitamin K was injected into the amniotic fluid. In the first case 10 mg were injected days before the delivery. In the second case 30 mg injected four days before the delivery.

Group IV In four cases the foetus was given an intramuscular injection of 2.5–3.0 mg phytonadion in utero.

A locally constructed ultrasonic multitransducer scanner was used (10). It operates with an array of 50 transducer units which are activated sequentially. The system yields real time cross sectional images of a 10 cm wide and 16 cm deep area consisting of 50 parallel lines. Through a special puncture adapter which can be fastened to one end of the transducer it is possible to introduce and manipulate a needle in the rectangular sound field (7).

Initially the buttocks of the foetus were located. Then under sterile conditions a guide needle 1.2 mm o.d. was passed via the puncture adapter through the abdominal and uterine walls aiming at the buttock. As the needle entered the sound field it became visible on the real time cross sectional scanning picture. Through the guide needle a long 0.6 mm o.d. needle was inserted into the uterine

cavity where the tip of the needle could be seen. Under direct monitoring the thin needle was advanced into the buttock of the foetus and the phytonadion was injected.

Before the intrauterine injection the mother received 5 mg vitamin K intravenously. Immediately after the injection of phytonadion the anticoagulant therapy was continued using intravenous heparin. The heparin was given by infusion controlled by the thrombin time, prothrombin time a.m. Quick and plasma recalcification time. The infusion of heparin was stopped few hours before delivery.

Immediately after the delivery the phenindione treatment was resumed by an injection of 100 mg phenindione followed by medication by mouth.

The newborn infant received 5 mg phytonadion intramuscularly as soon as blood was obtained for coagulation analyses.

Coagulation status on the newborn infants

The analyses were carried out on blood from the umbilical cord or—in two cases—from the scalp vein.

Factors II and VII were determined by the method of Owen & Aas (9). Factor IX by the method of Rodnol et al. (13) and the factor X according to Sise et al. (14).

CASE HISTORIES

Case 1 A B W 30 years old gravida III para II admitted to the hospital because of vaginal bleeding from the 17th week of pregnancy. Few days after the admission the patient developed a profound pelvic thrombophlebitis confirmed by phlebography (only a single picture was taken). Oral anticoagulant therapy was started using phenindione. When the expected time of delivery was reached the L/S ratio was determined. The L/S ratio was 1.5 and the estimated foetal weight was 2700 g. The foetus was given 3 mg phytonadion intramuscularly. Two days later labour was induced using a Cardiff infusion method. The mother was given 1 mg phytonadion and oral anticoagulant therapy was reestablished using heparin. The patient was delivered of a normal girl 1740 g, Apgar score 9/9. After the delivery the oral anticoagulant therapy was started again.

Case 2 E N 30 years old gravida IV para III admitted to the hospital during the 16th week of pregnancy with an ilio femoral popliteal thrombosis 7 weeks before the estimated time of delivery. The foetus weight was judged to 3000 g and the L/S ratio was 1.5. 5 mg phytonadion was injected into the foetus and anticoagulant therapy was changed from phenindione to heparin. The labour was induced two days later and a normal girl was born 3140 g, Apgar score 10/10.

Case 3 M L 34 years old gravida II para I admitted to the hospital in the 20th week of pregnancy with an acute abdomen. Appendicectomy was performed but the appendix was normal. Four days later the patient had a pulmonary embolism and anticoagulant therapy was initiated. Two weeks before the expected time of delivery the L/S ratio was 3.7 and the foetal weight was estimated to be more than 3500 g. 5 mg phytonadion was

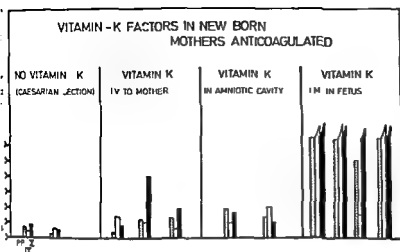


Fig 1 Coagulation factors in eleven infants born by mothers treated with anticoagulants. For each infant the values are indicated with three columns as percent of normal adult values. First column 1P%. Second column factor IX. Third column Factor X.

jected intramuscularly in the foetus. The mother was kept on a heparin infusion until the delivery 30 hours later. A normal boy 7800 g was born, the Apgar score III and the infant did not have any sign of haemorrhagic complications.

Case 4 K D H 25 years old primigravida was admitted during the fourth month of pregnancy because of a profound thrombosis of the left leg. Oral anticoagulant therapy was initiated. Two weeks before expected time of delivery the foetal weight was estimated to 3400 g. The foetus received 7.5 mg phytomenadion intramuscularly. The mother was given vitamin K intravenously and the anticoagulant therapy was continued with heparin. One day later labour was induced with oxytocin. During the second stage of labour serious decelerations in the cardiotocogram forced the obstetrician to apply a vacuum extractor. The patient was delivered of a healthy boy 36 hours after the injection of vitamin K. 3600 g. Apgar score 10. There was no sign of intracerebral haemorrhage.

RESULTS

Eleven mothers and their infants survived. One of the mothers who was delivered by caesarean section developed a pulmonary embolism but recovered.

Fig 1 illustrates the results of the coagulation analyses. All the coagulation factors were extremely low in the group in which the mothers did not receive vitamin K. Phytomenadion given to the mother a few days before the delivery did not improve the coagulation status significantly except for factor X in one of the cases. Intraamniotic injection of vitamin K few days before the delivery had the same unsatisfactory effect.

Intramuscular injection of vitamin K into the foetus *in utero* resulted in coagulation factors within

the normal (adult) range in three of the four infants. In case 3 the factors were significantly better than in the infants of the other groups. The factor X was normal but the PP% and factor IX were still reduced.

The injection marks were found in the gluteal region of the infants of group IV and the injections had caused no harmful effects.

DISCUSSION

The coagulation status of the infants of group I in which the mothers did not receive vitamin K before delivery confirms the assumption that the oral anticoagulant has passed the placental barrier. The infant is exposed to great risk of haemorrhage during the delivery even in case of caesarean section.

Injection of vitamin K to the mother (group II) did not improve the coagulation status for the newborn. Hirsh et al (6) introduced this method. Pridmore et al (12) treated 15 mothers with 5 mg of vitamin K seven to ten days before induction of labour. All the infants were normal and healthy and in the 12 tested the cord prothrombin times were within the normal range. However, the prothrombin time or prothrombin ratio is not suitable for the estimation of the vitamin K factors. It is not influenced by variations in factor IX and very little sensitive to factor X. Furthermore, the slightest contamination of gelatinous tissue from the umbilical cord alters the thromboplastin time significantly. In our study the factors II, VII, IX and X were studied and they were all very low.

Based on the literature and their own investiga-

tion Pridmore et al (12) suggest that the minimum time necessary for the effect of oral anticoagulants on the foetus to wear off is somewhere between three and fourteen days. In our study the longest time interval between the injection of vitamin K to the mother and the delivery was four days. This may explain why the coagulation status in these infants did not improve.

Injection of vitamin K into the amniotic fluid did not give any better results. In these cases the longest time interval between the injection and the delivery was four days also. Again the time may be too short to allow the foetus to resume a normal coagulation status.

The coagulation status of three of the infants which received an intramuscular injection of vitamin K *in utero* was normal. In only one case were the values subnormal. In that case the delivery took place 30 hours after the injection of vitamin K to the foetus. Therefore the time interval may have been too short. However the coagulation status was even in this case better than in the infants of the other groups.

It is possible that injection of vitamin K into the mother seven to ten days before the delivery may give as good results as the presented method of injecting the vitamin K into the foetus. However the administration of heparin is difficult and it is an advantage if the heparin treatment can be reduced to 48 hours. Using the L/S ratio the optimal time of induction of labour may be determined. The vitamin K can be given as soon as the L/S ratio is higher than 2.0 and the oral anticoagulant should be replaced by heparin. Labour can then be induced 48 hours later.

REFERENCES

- 1 Aaro L A, Johnson T R & Juergens J L. Acute deep venous thrombosis associated with pregnancy. *Obstet Gynecol* 28: 553 1966.
- 2 Bryant G M, Gray O P, Frazer A J & Ackerman A. *Br Med J* 1: 407 1970.
- 3 Causes of death in the Kingdom of Denmark 1975. National Health Service, Copenhagen 1975 p 10.
- 4 Cegelski F C, DeWeese J A & Lund C J. *Am J Obstet Gynecol* 89: 510 1964.
- 5 Engelberg H. Heparin Metabolism: physiology and clinical application. Thomas Springfield Ill 1974.
- 6 Hirsch J, Cade J F & O'Sullivan E F. *Br Med J* 1: 270 1970.
- 7 Holm H H, Pedersen J F, Knudsen J L, Rasmussen S N, Hancke S & Jensen F. Ultrasonically guided percutaneous puncture. *Radio North Am* 13: 493 1975.
- 8 Merz W R & Brextnier J. Wirkung der Drogen auf den Fetus. *Geburtsh Frauenheilk* 16: 426 1974.
- 9 Owren F A & Aas K. *Scand J Clin Invest* 5: 1951.
- 10 Pedersen J F & Northeved A. An ultrasound multitransducer scanner. *J Clin Ultrasound* 1975.
- 11 Pohl M & Kornhuber G. Fruchtschädigung und Antikoagulantienbehandlung in der Schwangerschaft. *Med Klin* 61: 964 1966.
- 12 Pridmore B R, Murray K H & Allen P M. The management of anticoagulant therapy during and after pregnancy. *Br J Obstet Gynaecol* 81: 740 1974.
- 13 Rodman N F, Barrow E M & Graham J B. *J Clin Pathol* 29: 525 1978.
- 14 Sise H S, Lavell S M & Becker R. *Proc Soc Exptl Biol Med* 96: 667 1957.
- 15 Shervington P C. Antenatal thrombosis and embolism. In: *Obstetric Therapeutics* (ed D F Hanks) p 243. Baillière Tindall, London 1974.
- 16 Thomson W N. *Am J Obstet Gynecol* 63: 313 1971.
- 17 Turnbull A C, Gwyn Daniel D & McGarry J M. Antenatal and postnatal thrombo-embolism. *Practioner* 206: 727 1971.
- 18 Villa Santa U. Thrombo-embolic disease in pregnancy. *Am J Obstet Gynecol* 97: 147 1965.
- 19 Yahr M D, Reich C & Eggers C. *Surg Gynecol Obstet* 80: 615 1945.

Submitted for publication Nov 18 1976

Jørgen Falck Larsen
Department of Obstetrics and Gynaecology
Herlev University Hospital
DK 2730 Herlev, Copenhagen
Denmark

CHANGES IN FETAL SUPRAVENTRICULAR EXTRASYSTOLES DURING UTERINE CONTRACTIONS IN LABOUR

Helge Jenssen

From the Department of Obstetrics and Gynecology
Aker Hospital (Oslo City Hospitals) Oslo, Norway

Abstract The electrocardiogram and phonocardiogram were recorded from a fetus exhibiting supraventricular ectopic beats during labour. The ectopic beats showed increased amplitude of the first heart sound (S_1) compared to sinus and postectopic beats. The $R-S_1$ interval was prolonged and the mechanical systole, measured as the S_1-S_2 interval, shortened. During uterine contractions these parameters were measured every 10 sec. The S_1 amplitude of the ectopic beats decreased simultaneously the $R-S_1$ interval increased and the S_1-S_2 interval shortened. The changes were delayed compared to the amniotic pressure curve. The changes found can be explained by a shift of blood between the fetus and placenta caused by uterine contraction.

Conspicuous changes related to uterine contractions were observed in the fetal phonocardiogram (FPCG) in a recording made during labour in which the fetal heart showed supraventricular ectopic beats (Fig. 1). An analysis of the FPCG and the simultaneously recorded fetal electrocardiogram (FECG) was undertaken measuring systolic time intervals (5). The arterial pulse was not recorded

extrasystoles. Several routine examinations by another pediatrician up to the age of 1 year showed normal development. The placenta was normal macroscopically and weighed 500 g.

METHOD

The FECG and FPCG were recorded during first stage of labour at a cervical diameter of 4-5 cm. Two hours earlier the mother had received diazepam (Stesolid "Dumex") 10 mg. During contractions she was hyperventilating to some degree breathing N₂O/O₂ 50/50% through an open mask. Amniotic pressure was registered as earlier described (7). The FECG was registered with a scalp spiral electrode; the signal was passed through a pre amplifier (EMT 1¹). The FPCG was recorded from the maternal abdomen with a heart sound microphone (EMT 25 B) and passed through a band pass filter with a nominal setting of 50 Hz and a pre amplifier (EMT 22). The signals were written on a Mingograph 34 continuously during the contractions; paper speed 100 mm/s. Measurements were made every 10 sec (s) starting 40 s before peak contraction pressure (Fig. 2).

The parameters measured are given in Table 1. As the Q wave varied to some extent $R-S_1$ interval was measured

CASE STUDY

The mother was 28 years old and gravida II. One and a half years earlier she had delivered a stillborn fetus in the 36th week. She was admitted in the 36th week because of arterial hypertension RR 140/100. Estriol excretion varied between 12 and 24 mg per 24 hours. Blood pressure fell to normal values within 24 hours, but she was kept in the ward for nearly 2 weeks chiefly due to anxiety caused by her previous stillbirth.

Four days after expected term she was readmitted without signs of preeclampsia in active labour and had an uneventful spontaneous delivery after 7 hours of contraction. The newborn, a boy, scored Apgar 8/10 at 1/5 min of age and weighed 3 630 g. On the first day of life a pediatrician found some extrasystoles but no other pathological findings. Three days later no extrasystoles were detected. An ECG of the newborn showed normal P waves and no

Table 1 Characteristics of sinus beats, ectopic beats and postectopic beats. Mean values between uterine contractions

$R-R$ time between the R waves of the actual and preceding beats. S_1 first heart sound, S_2 second heart sound

		Sinus beats	Ectopic beats	Postectopic beats
$R-R$ interval	ms	42	795	525
S_1-S_2 interval	ms	205	167	205
S_1-S_1 interval	ms	270	90*	370*
$R-S_1$ interval	ms	36	45	30
S_1 duration	ms	75	75	84
S_2 duration	ms	63	60	53
S_1 amplitude	mm	11	23	8
S_2 amplitude	mm	9	11	6

From S_2 of the preceding sinus beat to S_1 of the ectopic beat.

* From S_1 of the ectopic beat to S_1 of the postectopic beat.

¹ Manufacturer: Elema Schönder Stockholm

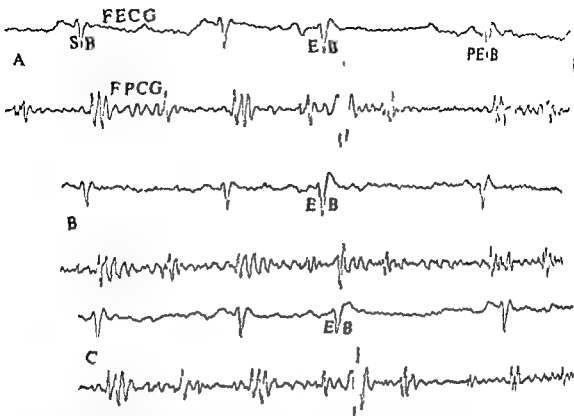


Fig 1 Fetal electrocardiogram (FECCG) and phonocardiogram (FPCG) recorded simultaneously A before uterine contraction B 10 seconds after maximal amniotic pressure (peak contraction pressure) and C 40

seconds after B SB sinus beats EB ectopic beats PE postectopic beats The FECCG was registered with downward deflection of the R wave

instead of $Q-S_1$ interval. The start of S_1 and S_2 was defined as the crossing point between the first high frequency vibration and the base line level. Using a magnifying lens and the smallest marks on the Mingograph paper readings were made to the nearest 2.5 millisecon (ms). Each value was the mean of 2-5 individual readings and the values presented are the means of 2 consecutive contractions.

RESULTS

Between uterine contractions

The FECCG trace was inverted (Fig 1). However between contractions the P waves were well delineated. The PQ time of the sinus beats was 60 ms of the ectopic beats 80-85 ms and of the postectopic beats 60 ms.

The amplitude of S_1 and S_2 was increased in the ectopic beats. The S_1-S_2 interval was shortened and the R- S_1 interval prolonged (Table 1). If the time of isovolumetric relaxation of the ventricles is disregarded the S_1-S_2 interval may be taken as a meas-

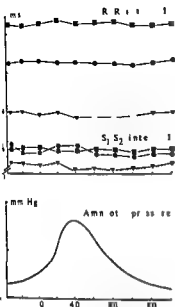
ure of ventricular filling time. The S_1-S_2 interval preceding the ectopic beats is 41% and that preceding the postectopic beats 168% of the sinus beats.

During uterine contractions

Fetal heart rate measured as the R-R interval did not vary during contractions (Fig 2). The S_1-S_2 interval of the ectopic beats shortened at peak contraction pressure, the shortening being maximal (10 ms) 20 s later and lasting 30-35 s.

The S_1 amplitude of the ectopic beats decreased 37% and of the sinus beats 19% during contractions (Figs 1 and 3). These changes too were delayed compared to the peak contraction pressure. The S_2 amplitude changed as shown in Fig 4.

The duration of the R- S_1 intervals in the ectopic beats (but not in the sinus or postectopic beats) increased during uterine contractions (Fig 4). The increase starting at the time of peak contraction pressure being maximal (27%) 10 s later and lasting 35-40 s.



2 Mean of $R-R$ interval and S_1-S_2 interval measured every 10 sec shown in relation to the amniotic pressure. \circ — \circ sinus beats ∇ — ∇ ectopic beats \blacksquare — \blacksquare ectopic beats ms milliseconds s seconds

The duration of S_2 of the sinus and ectopic beats is 75% shortened simultaneously with the changes in S_1 amplitude and $R-S_1$ interval. The duration of S_2 of the postectopic beats did not then

DISCUSSION

Fetal ectopic beats resemble the supraventricular extrasystoles of the human adult in its altered P wave configuration, increased S_1 amplitude and prolonged $R-S_1$ interval (2). The compensatory S_1-S_2 interval was not complete and the ventricular conduction of the ectopic beats was virtually unchanged. The ectopic beats of the present case is considered to have a supraventricular origin. The duration of $R-S_1$ and S_1-S_2 intervals of the ectopic beats are the same as reported by others (3).

The pressure increase during uterine contraction is too small to influence the velocity of propagation of the fetal heart sounds to a measurable extent. The $R-S_1$ interval of the ectopic beats increases with the peak contraction pressure simultaneously S_1 amplitude decreases. Disappearance of the initial

deflection of S_1 accompanying decrease of amplitude cannot explain the $R-S_1$ interval change as the duration of S_1 did not change. The sum of $R-S_1$ and S_1 duration increased during contraction in the same manner as the $R-S_1$ interval.

The delay of S_1 in the ectopic beats is accompanied by delay in central arterial pressure increase in systole (14) and may be partly caused by the increased aortic pressure at the start of the ectopic beats. If the duration of the S_1-S_2 interval preceding the ectopic beats is applied to the aortic pressure curve recorded in the newborn (13) aortic pressure at the start of the ectopic beats systole is still about 35% of maximal aortic pressure. In hypertensive adults prolonged $Q-S_1$ intervals have been found (15).

Among causes of a loud first heart sound Leatham lists reduced ventricular filling from atrioventricular stenosis or tachycardia (9). In dogs Sakamoto et al (11) showed that among several factors influencing S_1 amplitude the only common factor was the rate of left ventricular pressure increase—a rapid pressure increase giving a high S_1 amplitude. Decreased ventricular filling in the ectopic beats therefore explains the high S_1 amplitude.

Shortening of the S_1-S_2 interval in the ectopic beats may be caused by increased aortic pressure or reduced cardiac filling.

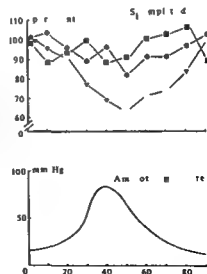


Fig 3 Relative changes of S_1 amplitude related to uterine contraction. Symbols as in Fig 2. The values of Table 1 taken as 100%.

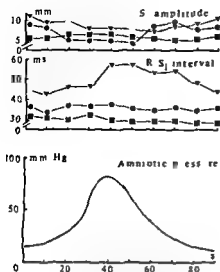


Fig 4 Changes of S_2 amplitude and $R-S_1$ interval duration related to uterine contraction. Symbols as in Fig. 2

During uterine contraction the intervillous circulation is partly or totally stopped (1). Peak contraction pressure of the contractions studied was 60 to 70 mmHg, probably occluding intervillous circulation. During amniotic pressure rise there must be a period of intervillous space increase as the veins draining the intervillous space are occluded before the arteries.

The intervillous space increase may have an effect on the fetal circulation. A bolus of blood may be squeezed from the placenta to the fetus temporarily increasing the return of blood to the fetal heart. Resistance in the chorionic tree may increase causing elevation of the fetal arterial pressure. These circulatory changes may explain the principal variations during uterine contraction found in the present case (change of the S_1 amplitude and the $R-S_1$ and S_1-S_2 intervals).

A 10% reduction of newborn's predicted total blood volume during exchange transfusion (4) did not alter the $R-S_1$ interval but significantly shortened the $R-S_2$ interval and consequently the S_1-S_2 interval. However, exact timing of S_1 was inaccurate due to small amplitude.

Khanna et al. (8) reduced stroke index in adults with peripheral cuffs. This procedure prolonged the pre-ejection period and shortened left ventricular ejection time. Presuming that the $R-S_1$ interval partially reflects pre-ejection period changes (6) and that the S_1-S_2 interval reflects changes of left ventricular ejection time (5) the changes related to

uterine contractions are mainly caused by change in return of blood from the placenta.

The present case shows that the fetal's time intervals has the ability to change much like the adult and that the changes are measurable. These findings add support to the hypothesis that a shunt of blood between the fetus and placenta takes place during uterine contraction.

REFERENCES

1. Borell U, Fernstrom I, Ohlsson L & Wapner H. An arteriographic study of the blood flow through the uterus and the placenta in midpregnancy. *Acta Gynecol Scand* 44: 27, 1965.
2. Cossio P, Dambrosi R G & Warnford-Tex H F. The first heart sound in auricular and ventricular extrasystoles. *Br Heart J* 9: 775, 1947.
3. Craige E & Harned H S. Phonocardiographic and electrocardiographic studies in normal newborn infants. *Am Heart J* 65: 180, 1963.
4. Gyulai F & Walsh S Z. Phonocardiographic studies during experimental hypo- and hypernatremia in the healthy newborn. *J Electrocardiol* 4: 149, 1973.
5. Harris L C, Weissler A M, Manske J, Danford B H, White G D & Hamill W A. Relation of the phases of mechanical systole in normal children. *Am J Cardiol* 14: 448, 1964.
6. Harris W S. Systolic time intervals in the noninvasive assessment of left ventricular performance. In: *Cardiac Mechanics: Physiological and Mathematical Considerations* (ed. by N. Ghista & H. Sandler) p. 744. Wiley, New York, 1974.
7. Jenssen H. The effect of paracervical block on cervical dilatation and uterine activity. *Acta Gynecol Scand* 52: 13, 1973.
8. Khanna P K, Shah P B, Kramer D H, Shetty R A & Tager I. Effects of altered preload on ventricular systolic time intervals in acute myocardial infarction. *Br Heart J* 35: 1107, 1973.
9. Leatham A. *Auscultation of the Heart and Percussion* p. 26. J. & A. Churchill, London, 1970.
10. Malecki I. *Physical Foundations of Test Acoustics*. Pergamon Press, Oxford, 1969.
11. Sakamoto T, Kusukawa R, MacCannell D I, Luisada A A. Hemodynamic determinants of the amplitude of the first heart sound. *Circulation* 33: 11, 1965.
12. Walsh S Z & Gyulai F. Electrocardiographic studies in the healthy newborn. *J Electrocardiol* 4: 170, 1970.
13. Walsh S Z & Lind J. The dynamics of the heart and circulation and its alteration at birth. *Physiology of the Perinatal Period* (ed. by J. Stave) p. 183. Appleton-Century-Croft, New York, 1970.

Weissel W & Vetter H. Herzkatheteruntersuchungen des Verhaltens der elektro-pressorischen Latenz bei Rhythmusstörungen. *Cardiologia* 20: 160 195

Weissler A M, Leonard J J & Warren J V. Observations on the delayed first heart sound in mitral stenosis and hypertension. *Circulation* 18: 165 1958

Submitted for publication March 3 1977

Helge Jenssen
Department of Obstetrics and Gynecology
Aker Hospital
Oslo
Norve

THERAPEUTIC ABORTION

The 1975 report from Ullevål Hospital

Fritjof Jerve and Petter Fylling

From the Department of Obstetrics and Gynecology Ullevål Hospital Oslo Norway

Abstract The results from a prospective study of 1228 therapeutic abortions (the 1975 material) are reported. All trimester pregnancies (1078 cases) were terminated by suction and curettage and second trimester pregnancies by prostaglandins (200 cases). The overall follow-up (4-6 weeks after termination) was 94.3%. The incidence of abortion was 0.5% of the surgical terminations; none of which however resulted in any other complications. The incidence of re-admission was 3.9%, the main causes being retained products or pelvic infection. The incidence of pelvic infection (salpingitis/parametritis) was 1.6%. The highest incidence of pelvic infection was found in early pregnancies (≤ 8 weeks): nulliparous women (7.7%) and the lowest (1.5%) in the induction group.

Key words: complications associated with therapeutic abortion have gradually decreased during the last decade. This trend is concurrent with the increase in the number of therapeutic abortions performed, improvement of the techniques and greater experience and training of medical personnel in this special field (2, 11, 13, 18, 19, 22, 23). Hence prospective studies for limited periods and a certain homogeneity of the staff involved should give a more reliable picture of the current situation in each centre or region.

Our 1975 material on therapeutic abortion, based on recording each step of the treatment procedure on EDB forms specially designed for this purpose, is reviewed.

MATERIAL AND METHODS

The series consists of 1228 pregnancies that were terminated at our clinic in 1975. All women underwent a gynecological examination at our outpatient clinic 1-7 days prior to the termination. The majority of the women were examined by one of us (P.F.). At this initial visit the medical history was recorded and the size of the uterus estimated. Samples for the relevant laboratory tests such as Rh etc. were also secured at this visit.

Based on the size of the uterus the women were divided into 2 groups. The first group included nulliparous women ≤ 11 weeks pregnant and parous women ≤ 12 weeks pregnant. The second group included all second trimester pregnancies and also nulliparous women 12 weeks pregnant. The first group was treated on an outpatient basis by a surgical method. A few patients in this group were admitted to hospital for simultaneous sterilization or because of pregnancies complicated with medical disorders such as diabetes etc. The second trimester pregnancies were terminated by induction.

Surgical procedure The women attended the outpatient clinic in fasting condition at 8 o'clock a.m. and the terminations were performed under i.v. anaesthesia by cervical dilation and vacuum aspiration. As a general rule a vacuum metal aspiration curette No. 8 was used up to 9-10 weeks and No. 10 after 9-10 weeks. The women were observed 3-4 hours postoperatively and then discharged from the hospital.

Induction of abortion All inductions were performed by prostaglandins. Different methods were used as parts of clinical trials administered by the Prostaglandin Task Force, Karolinska Institutet, Stockholm.

Eighteen women underwent sterilization simultaneously with the termination (4 by laparotomy and 14 by laparoscopy).

Vaginal bacteriological screening was not performed as a routine. No pregnancies were terminated by hysterotomy and Gamaglobulin was given to all Rh negative women.

Follow-up On discharge from the hospital all women were given an appointment for a follow-up visit and written instructions which emphasized the importance of control. If a woman failed to keep this appointment about 4 weeks later she received a new one by mail. If she still did not appear she was given a third chance by filling in a questionnaire which was sent to her by mail.

The data recorded were handled by the municipal EDB-department.

RESULTS

The distribution of the material according to age groups is illustrated in Fig. 1. As many as 24.2% of

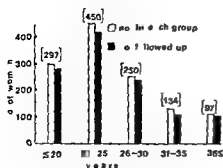


Fig 1 Distribution of the material in age groups. The total number in each group is indicated in the parentheses

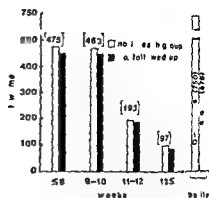


Fig 2 Distribution of the material in gestational length at parity. The total number in each group is indicated in the parentheses

the women were less than 21 years old and 36.4% were between 21 and 25 years old.

As shown in Fig. 2 the majority of the pregnancies (76.4%) were terminated within the first 10 weeks of gestation and 61.1% of the women were nulliparous.

The main peroperative complications are listed in Table 1, but none of them were serious. None of the 5 uterine perforations, i.e. 0.5 per cent of the surgical terminations, resulted in any other immediate or late (4-6 weeks) complications. Patients with uterine perforations were observed in the hospital overnight and then discharged. Although the figures are small, they might indicate a certain percentage increase in the incidence of perforations with increasing gestational length.

As may be expected, a higher incidence of cervical laceration was observed in the nulliparous group. However, the lacerations were always of minor degree and did not necessitate any treatment. The bleeding recorded in 12 patients was also of moderate degree and blood transfusion was given in only one case, a prostaglandin-induced abortion.

Fig. 3 shows the incidence of re-admission or pelvic infection related to age groups or parity. By far the highest incidences of both re-admission and

pelvic infection were found among young (<21 years) or nulliparous women.

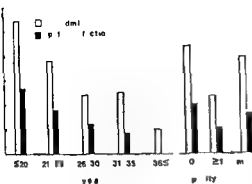
Fig. 4 illustrates the incidence of re-admission or pelvic infection related to gestational length and methods used. Contrary to what was expected, the percentage highest incidence of pelvic infection was found in the ≤8 weeks group while in the induction group (200 cases) only 3 cases were observed. These 3 cases were nulliparous women and one of them was 12 weeks and the 2 others were 13 and 14 weeks pregnant.

The percentage re-admission seems to be a fairly good indicator of the quality of the technical procedure. In Table II the percentage of re-admission related to the different medical officers is illustrated. The clinical and technical experience of the medical officers A-C was greatest and that of G-I was least. Bleeding, pain or fever were the most common symptoms causing re-admission.

Follow-up: 1108 or 90.2% of the women appeared at the follow-up visit and 60 replied to questionnaires. Hence we obtained postoperative information on totally 94.3% of the material. The postoperative period varied from 4 to 8 weeks, mostly 4 to 5 weeks.

Table I. Peroperative complications related to gestational length or parity

	Gestational length in weeks				Parity		Total
	≤8	9-10	11-12	≥13	0-Parous	Parous	
Perforation	3 (0.6%)	1 (0.2%)	1 (1.0%)	0	4 (0.6%)	1 (0.2%)	5 (0.4%)
Laceration of the cervix	1 (0.2%)	1 (0.2%)	0	1 (1.0%)	3 (0.4%)	0	3 (0.2%)
Bleeding	2 (0.4%)	3 (0.6%)	5 (2.6%)	0	5 (0.7%)	5 (1.0%)	10 (0.8%)



3 The incidence of re-admission or pelvic infection (salpingitis/parametritis) related to age groups or parity

DISCUSSION

The present material is similar to some previous reports concerning age and parity (3, 15, 19, 23). Intravenous administration of Brietal Natrium[®] was found to be a very suitable anaesthesia for this type of surgery. We recorded a low percentage of discomfort among patients shortly after the procedure. Although we are aware of the widespread use of local anaesthesia for this type of surgery (1, 9, 15, 19) at least in our hands intravenous anaesthesia seems to be preferable because of the safety for the procedure usually experienced by women in our population.

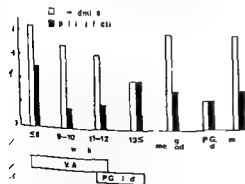
We found relatively few perioperative complications in the present material. The figures for perforation (0.5%) are higher than those reported by others (6, 12, 16, 17, 19) but none of the perforations caused any additional damage. Three

out of the 5 perforations occurred in cases of early pregnancy (≤ 8 weeks). It is reasonable to assume that this complication at least in the 8 weeks group could be avoided by using Karman cannulae (10, 15). On the other hand a higher incidence of retained products is reported using plastic cannulae compared with metal ones (3).

Exact quantitation of perioperative bleeding was not performed in the present series. Bleeding more than usual was recorded as 'bleeding'. In only one case a second trimester abortion induced by prostaglandins the bleeding necessitated a blood transfusion.

Other perioperative complications were also relatively few, especially in the second trimester group compared with some previous reports (2, 6-8, 11, 13, 19, 22, 23). As already reported by several investigators reviewed by Brenner (4) induction of second trimester abortion by prostaglandins is superior to other methods for termination. Hence surgical termination of pregnancies beyond the 12th week should be avoided. However the intermediate group (10-11 weeks) might still be terminated by vacuum aspiration eventually combined with preoperative prostaglandin treatment.

The figures for post termination complications will obviously be dependent on 1) the criteria used in the assessment of complications, 2) the evaluation techniques used, and 3) the observation period. The highest incidence of salpingitis/parametritis was observed in the ≤ 8 weeks group and this was entirely unexpected. At least 3 possibilities exist: (a) it is more difficult to empty the cavity completely in this group, (b) the medical officer may consider the procedure to be easier in this group and hence might not be vigilant enough during the vacuum aspiration procedure, (c) some factors like high sexual activity causing a higher prevalence of pelvic infections in this group. Of a total of 20 cases with pelvic infection in the ≤ 8 group as many as 17 were nulliparous. Hence in the authors' opinion a combination of causes is a reasonable explanation.



4 The incidence of re-admission or pelvic infection (salpingitis/parametritis) related to gestational length and methods used. V.A = vacuum aspirations, P.G. = prostaglandin induced abortions

Table II Percentage re-admission per medical officer

Medical officer	A	B	C	D	E	F	G	H	I
Re admission	30	32	33	40	42	47	48	50	59

for the higher incidence of pelvic infection in early pregnancy

The policy has been to terminate the pregnancies as early as possible as this has proved beneficial both from a psychological and somatic point of view (14-19, 20). Since the present methods for terminating early pregnancies at least in our hands do not appear to be so satisfactory as anticipated greater efforts must now be made to improve these procedures.

ACKNOWLEDGEMENT

This investigation received financial support from the World Health Organization.

REFERENCES

- 1 Bendel R P, Williams P P & Butler J C. Endometrial aspiration in fertility control. A report of 500 cases. *Am J Obstet Gynecol* 125: 328, 1976.
- 2 Berthelsen H G & Østergaard E. Techniques and complications of therapeutic abortion. *Danish Med Bull* 6: 105, 1959.
- 3 Borko K, Breznik R, Kokos Z, Edelman D & Brenner W. First trimester abortion by vacuum aspiration. *Ann Chir Gynaecol Fenniae* 64: 320, 1975.
- 4 Brenner W E. The current status of prostaglandins as abortifacients. *Am J Obstet Gynecol* 123: 306, 1975.
- 5 Brenner W E, Edelman D A & Kessel E. Menstrual regulation in The United States. A preliminary report. *Fertil Steril* 26: 289, 1975.
- 6 Edelman D A, Brenner W E & Berger G S. The effectiveness and complications of abortion by dilatation and vacuum aspiration versus dilatation and rigid metal curettage. *Am J Obstet Gynecol* 119: 473, 1974.
- 7 Fylling P & Refsdal A. Rivanol induced mid trimester abortion. *Arch Gynak* 215: 359, 1973.
- 8 Fylling P & Refsdal A. Therapeutic abortion by a single extra amniotic instillation of prostaglandin $F_{2\alpha}$. *Arch Gynak* 217: 119, 1974.
- 9 Golditch I M & Glasser M H. The use of laminaria tents for cervical dilatation prior to vacuum aspiration abortion. *Am J Obstet Gynecol* 119: 9, 1974.
- 10 Johnstone F D, Beard R J, Boyd I E & McCarthy T G. Cervical diameter after suction termination of pregnancy. *Br Med J* 68: 1976.
- 11 Kerenyi T D, Mandelman N & Sherman D R. Five thousand consecutive saline inductions. *Am J Obstet Gynecol* 116: 593, 1973.
- 12 Kerslake D. Abortion induced by means of a uterine aspirator. *Obstet Gynecol* 30: 35, 1967.
- 13 Kolstad H. Therapeutic abortion. *Acta Obstet Gynecol Scand* 34: Suppl. II, 1957.
- 14 Lebensohn Z M. In: *Abortion Techniques and Service* (ed S Lewit) p. 55. Excerpta Medica, Amsterdam, 1972.
- 15 Lewit S C, Lal S, Branch B & Beard R W. Outpatient termination of pregnancy. *Br Med J* 1: 1971.
- 16 Moberg P J. Uterine perforation in connection with vacuum aspiration for legal abortion. *Int J Gynaecol Obstet* 14: 77, 1976.
- 17 Nathanson M. Management of uterine perforations suffered at elective abortion. *Am J Obstet Gynecol* 119: 473, 1974.
- 18 Potts D M. Termination of pregnancy. *Br Med J* 26: 65, 1970.
- 19 Rovinsky J J. Abortion in New York City. Preliminary experience with a permissive abortion statute. *Obstet Gynecol* 38: 333, 1971.
- 20 Russel K P. In: *Abortion Techniques and Service* (ed S Lewit) p. 12. Excerpta Medica, Amsterdam, 1972.
- 21 Spivak M M. Therapeutic abortion. A 15 year view at the Toronto General Hospital 1954-1969. *Am J Obstet Gynecol* 97: 316, 1976.
- 22 Stewart G K & Goldstein P. Medical and surgical complications of therapeutic abortions. *Obstet Gynecol* 40: 539, 1972.
- 23 Tietze C & Lewit S. In: *Abortion Techniques and Service* (ed S Lewit) p. 47. Excerpta Medica, Amsterdam, 1972.

Submitted for publication Febr. 16, 1977

Fritjof Jerve
Department of Obstetrics and Gynecology
Ullevål Hospital
Oslo
Norway

EFFECT OF FASTING ON BLOOD GLUCOSE OF PARTURIENT AND HER FULL TERM INFANT

R S Ikonen E Hagman and P Pystynen

From the Departments of Paediatrics and Obstetrics and Gynaecology Central Hospital of Tampere and the Institute of Clinical Sciences University of Tampere Tampere Finland

Abstract The series consists of 100 parturients and their term infants. According to the fasting time of the parturients before delivery the series was divided into three groups: Group I: fasting from 6 to 12 hours (49 parturients), Group II: fasting from 12 to 18 hours (38 parturients) and Group III: fasting over 18 hours (13 parturients). The blood glucose level of the parturients was examined immediately after delivery and that of their infants at birth and at the age of two and six hours. There were no significant differences between the blood glucose level of the mothers in different fasting groups: 5.0 ± 0.8 mmol/l in Groups I and II and 4.9 ± 0.9 mmol/l in Group III. There was a significant positive correlation between the blood glucose level of the parturients at birth and that of their mothers ($r = 0.44$, $p < 0.001$). The blood glucose level of the infants at the age of two and six hours did not correlate to that of their mothers. Blood glucose of the infants was lowest at the age of two hours (2.5 ± 0.6 mmol/l) and there was no significant difference between the groups at any age. Hypoglycaemia (blood glucose less than 1.7 mmol/l) was found in 15 cases (15%). Four were treated because of symptomatic hypoglycaemia.

Prolonged fasting of parturients during labor and delivery is not unusual. We decided to investigate fasting in labour when we noticed that many infants had very low blood glucose values soon after birth. In our hospital women were given no food and only water to drink when they were in active labor.

Table 1. Fasting groups

Groups of parturients	No of parturients	Fasting time (h) (mean \pm 1 S D)
Group I (6-12 hours)	49	9.4 ± 2.5
Group II (12-18 hours)	38	14.6 ± 1.3
Group III (over 18 hours)	13	19.7 ± 1.9

MATERIAL AND METHODS

The study consists of 100 parturients who delivered normally at term. The parturients were divided into three groups according to the fasting time before delivery (Table I): Group I: fasting time from 6 to 12 hours (49 parturients), Group II: fasting time from 12 to 18 hours (38 parturients) and Group III: fasting time over 18 hours (13 parturients). By the term fasting time was understood the interval between the last meal (according to information obtained from the parturients) and the delivery.

The blood glucose level of the mothers was estimated by taking a capillary blood sample immediately after delivery and that of the infants at birth and at the age of two and six hours. Blood glucose was determined by glucose oxidase method (Kabi Technicon Auto Analyzer II). The infants were given 5% glucose at the age of two hours and thereafter were breast fed six hourly with additional glucose when necessary.

There was no significant difference between the groups in infants' gestation age, birth weight and condition at birth as estimated by one minute Apgar Scoring (Table II). The mean gestation age was slightly over 40 weeks, birth weight about 3 500 g and Apgar score 8.8.

RESULTS

There was no significant difference between the mean blood glucose level of the mothers in different

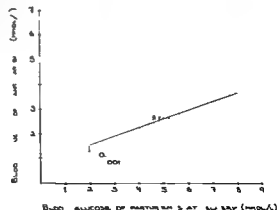


Fig. 1. Correlation between blood glucose of infants at birth and parturients at delivery.

Table II Gestation age birth weight and 1 min Apgar score distribution of infants in the different groups

Fasting groups	Gestation age (weeks) (mean \pm 1 S D)	Birth weight (g) (mean \pm 1 S D)	1 min Apgar score (mean \pm 1 S D)
Group I	40.4 \pm 1.3	3 610 \pm 520	8.9 \pm 0.6
Group II	40.2 \pm 1.4	3 540 \pm 480	8.8 \pm 0.7
Group III	40.1 \pm 1.0	3 410 \pm 370	8.8 \pm 0.7

Table III Blood glucose of parturients at delivery and infants at birth and at two and six hours age
In parentheses the number of cases in which blood glucose was not estimated

Fasting groups	Blood glucose of parturients (mmol/l) (mean \pm 1 S D)	Blood glucose of infants (mmol/l)		
		At birth	Aged 2 h	Aged 6 h
Group I	5.0 \pm 0.8 (1)	2.5 \pm 0.6	2.5 \pm 0.5 (?)	2.8 \pm 0.6 (9)
Group II	5.0 \pm 0.8 (2)	2.6 \pm 0.7 (1)	2.5 \pm 0.6	2.7 \pm 0.5 (4)
Group III	5.2 \pm 0.9	2.7 \pm 0.5	2.3 \pm 0.5	2.8 \pm 0.5 (1)

Table IV Incidence of hypoglycaemia in infants
(blood glucose less than 1.7 mmol/l)

At birth	Aged 2 h	Aged 6 h	All
6	7		13
1	1		1
	1	1	1
Treated because of hypoglycaemia			4

Table V Blood glucose of hypoglycaemic infants compared to that of their mothers at delivery

Infants at birth	Their mothers	Infants aged 2 h	Their mothers
1.6	5.6	1.6	5.6
1.6	5.2	1.6	5.2
1.5	4.0	1.6	4.8
1.4	4.7	1.6	4.6
1.2	3.8	1.4	6.0
1.2	3.8	1.4	5.6
1.2	-	1.4	5.2
		1.3	6.3
		1.0	5.4

fasting groups (Table III) 5.0 \pm 0.8 mmol/l in Groups I and II and 5.2 \pm 0.9 mmol/l in Group III. These values are quite high but within the normal range for adults.

No significant difference was also found between

the mean blood glucose level of the infants. Groups I, II and III at any age. The mean blood glucose was lowest at the age of two hours (2.5 mmol/l).

Although there was no difference between the blood glucose values in various fasting groups, there was a statistically significant positive correlation between the blood glucose of infants at birth and that of their mothers at delivery (Fig. 1).

The blood glucose level of the infants at the age of two and six hours did not correlate with that of their mothers (Figs. 2 and 3).

The incidence of hypoglycaemic values in the

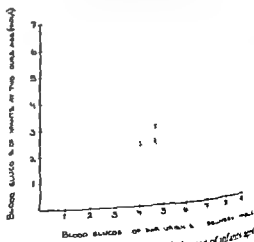


Fig. 1 Correlation between blood glucose of infants at 2 h and parturients at delivery.

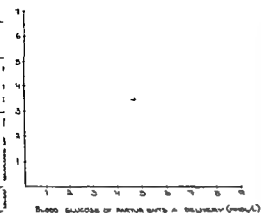


Fig. 3 Correlation between blood glucose of infants aged 1 h and parturients at delivery

ormally delivered full term infants was 15% (Table V). Blood glucose below 1.7 mmol/l was considered hypoglycaemic. In seven cases there was a hypoglycaemic value at birth and in eight cases at the age of two hours. In one case there was a hypoglycaemic value both at birth and at the age of 40 hours and in one case at the age of two and six hours. Four infants were treated because of symptomatic hypoglycaemia.

When comparing the blood glucose level of hypoglycaemic infants to those of their mothers there was found a correlation between the blood glucose of the infants at birth and that of their mothers at delivery but not later (Table V).

DISCUSSION

In earlier investigations (1-9) it has been shown that blood glucose of the fetus and the newborn at birth correlates with blood glucose of the mother and that maternal hypoglycaemia can cause fetal hypoglycaemia (8). It has also been postulated that blood glucose values of parturients vary depending on the time of the last meal (3). In contrast in this study there was no correlation between the fasting time of parturients and their blood glucose level and that of their newborn infants. The explanation of this may be increased corticosteroid production caused by the stress of labor and delivery to the parturient.

Blood glucose of infants was lowest at the age of two hours which is in agreement with earlier

studies (3-7). Cornblath et al. (2) have reported an appropriate for gestational age infants blood sugar values of less than 30 mg/100 ml in 13.7% of the cases during the first six hours. In Lubchenco & Bard's series (6) the incidence of blood sugar values of less than 30 mg/100 ml was 11.4% in a general nursery population before the first feedings at six hours of age. In this study hypoglycaemic values (blood glucose less than 1.7 mmol/l which corresponds 30 mg/100 ml) occurred in 15% of the cases during the first two hours and was present in half of the babies at birth. Later there was no new cases of hypoglycaemia perhaps because of early feeding.

The concept of hypoglycaemia in the newborn has changed in recent years and the classification has been revised (4). The greatest incidence of hypoglycaemia has been found to be immediately after birth (4) as in this study. Perinatal asphyxia is possibly one important factor in its development (4, 5, 6). In this study the infants were born in good condition but even then the incidence of low blood glucose values was 15%. In most the situation was corrected without any special management. With babies at risk screening of blood glucose should be initiated during the first hours after birth and early feeding used to prevent hypoglycaemia.

REFERENCES

- 1 Coltart T M, Beard W, Turner R C & Oakley H W. Blood glucose and insulin relationship in the human mother and fetus before onset of labor. *Br Med J* 4 17 1969.
- 2 Cornblath M, Ganzon A F, Nicolopoulos D, Baens G S, Holander R J, Gordon M H & Gordon H H. Studies of carbohydrate metabolism in the newborn infant. III. Some factors influencing the capillary blood sugar and the response to glucagon during the first hours of life. *Pediatrics* 27 378 1961.
- 3 Cornblath M & Schwartz R. Disorders of Carbohydrate Metabolism in Infancy. W B Saunders Company Philadelphia and London 1966.
- 4 Cutlerlet L & Cornblath M. Neonatal hypoglycemia revisited 1975. *Pediatrics* 58 10 1976.
- 5 Ikonen R S. Apgar scoring and neonatal morbidity in full sized newborn infants. *Ann Clin Res* 5 380 1973.
- 6 Lubchenco L O & Bard H. Incidence of hypoglycemia in newborn infants classified by birth weight and gestation. *Pediatrics* 47 831 1971.
- 7 McCann M L, Adam P A J, Likly B F & Schwartz R. Prevention of hypoglycosemia by fructose in infants of diabetic mothers. *N Engl J Med* 275 8 1966a.

- 8 Phillips L Lumley J Paterson P & Wood C
Fetal hypoglycemia *Am J Obstet Gynecol* 107 371
1968
- 9 Spellacy W N Suhi W C Bradley B & Holsinger
K K Maternal fetal and amniotic fluid levels of
glucose insulin and growth hormone *Obstet Gynecol*
41 323 1973

Submitted for publication March 12 1977

■ S Ikonen
Department of Paediatrics
Central Hospital of Tampere
33520 Tampere 52
Finland

CAESAREAN SECTION

A clinical study with special reference to the increasing section rate

Eva Patek and Bertil Larsson

From the Department of Obstetrics and Gynecology Huddinge Hospital Huddinge Sweden

Abstract At Huddinge University Hospital 539 Caesarean sections (C S) were made among 8415 deliveries from October 1972 to June 1976 corresponding to an over all C S rate of 6.4%. Over these years the rate has increased from 3.5% in 1972 to 9.7% in 1976. The main increase was due to a higher incidence of abdominal deliveries in cases of imminent fetal asphyxia. The maternal complication rate and the neonatal morbidity rate were both 6.5 times higher and the neonatal mortality rate was 4.1 times higher in emergency than in elective surgery. There was neither maternal mortality nor any morbidity in infants delivered by elective C S from healthy mothers at term.

Recently an increased rate of C S was reported not only in Sweden but also in USA and West Germany (5-7). With the growing emphasis on the prenatal and intrapartum status of the fetus and with the addition of laboratory tests and technical progress of internal fetal monitoring, an increased rate of C S should be expected (1). However, there must be an optimal rate of C S in which the maternal risks are in balance with the benefits of the fetus-child. In a recent paper it was pointed out that C S should still be regarded as the most dangerous way of delivery, not only for the mother but also for the child (7).

The aim of the present study was to evaluate the maternal risks in a series with an increasing rate of C S and make comparisons of the complications between emergency and elective C S associating them with the indications. Studies were also made of neonatal mortality and morbidity during these conditions.

FREQUENCY PARITY AGE

At Huddinge University Hospital 539 C S (6.4%) were made among 8415 deliveries from October

1972 when the hospital was opened through June 1976 (Fig. 1). As shown in Fig. 1 there was a threefold increase in the frequency of C S during this almost four year period. The number of children delivered by C S was 545 including four sets of twins and one set of triplets.

In more than half of the patients (57.9%) Caesarean delivery was performed on primiparae. Several patients were sectioned twice or thrice and two four times. The patients with C S were 16-46 years of age with a predominance of 21-35 years (85.5%).

INDICATIONS

Indications for the 525 C S performed from 1973-June 1976 are summarized in Table I. Only the main indication for each operation was recorded. Patients with a pelvic outlet of less than 29.5 cm or between 29.5-31.5 cm (borderline) after X ray pelvimetry were summarized as cephalopelvic dis-

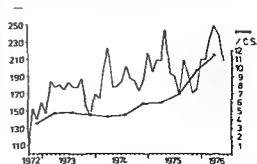


Fig. 1 Number of deliveries every month from October 1972-June 1976 at Huddinge University Hospital. C S ratio in % calculated three times per year.

Table I Indications for C S in % per year at Huddinge University Hospital 1973-June 1976

	1973 %	1974 %	1975 %	1976 %
Vertex & cephalopelvic disproportion	37.9	27.0	17.5	25.4
Breech & cephalopelvic disproportion or preterm	15.5	12.2	17.5	18.0
Fetal distress	9.7	14.8	22.8	26.1
Placenta praevia	9.7	2.6	3.5	3.7
Abruptio placentae	1.9	4.3	5.8	3.7
Prolapsed umbilical cord	-	2.6	3.5	-
Diabetes mellitus	1.9	3.5	1.2	-
Toxaemia of pregnancy	4.9	7.0	3.5	4.5
Malpresentation	-	3.2	1.8	1.5
Uterine anomaly or uterine scar	1.0	2.6	1.2	0.7
Fetal growth retardation	-	-	1.8	1.5
Dysfunction of placenta	1.0	3.5	4.7	1.5
Serious maternal disease	2.9	-	1.8	2.2
Obstetrically old primipara	2.9	3.5	4.7	3.7
Psychiatric indication	2.9	0.9	1.8	2.2
Bad obstetrical history	1.0	2.6	2.3	0.7
Imminent uterine rupture	1.0	0.9	-	1.5
Previous corporal longitudinal incision	1.0	-	0.6	0.7
Uterine inertia	4.9	3.5	3.5	1.5
Miscellaneous	1.0	-	0.6	0.7

proportions (3). The same diagnosis incorporated a true conjugate of the pelvic inlet of less than 9.5 cm or a conjugate of 9.5-11 cm (borderline). Breech presentation delivered by C S was always combined with either cephalopelvic disproportion or prematurity. Transverse lie, mentum posterior and face posterior presentations are included under the heading of malpresentations. Multiparity with a history of several intrauterine fetal deaths was registered as 'bad obstetrical history' as were cases with previous complicated deliveries.

Table II Complications during elective and emergency C S at Huddinge University Hospital October 1972-June 1976 Neonatal mortality Neonatal morbidity

	Elective C S n=241=44.7% n of infants=241 (%)	Emergency C S n=298=55.3% n of infants=304 4 twins 1 triplet (%)
Complications		
(a) Intra-operative	0.8	0.7
(b) Post-operative		
I Major complications	0	3.4
II Minor complications	2.5	13.1
	2.5	16.5
Neonatal mortality	0.8	3.3
Neonatal morbidity	1.7	10.9

OPERATIVE PROCEDURE

Induction of anaesthesia was made with a small dose of propanid (Eponal BAYER) followed by endotracheal intubation and maintenance of anaesthesia with nitrous oxide and oxygen plus neurolept (Dridol LEO). Muscular relaxation was achieved by an intravenous dose of succinylcholine (Celocuron VITRUM). Epidural anaesthesia was given 10 times.

A low midline incision was made in all women except in 67 (12.4%) where the Pfannenstiel method was employed. The uterus was entered through a transverse lower segment incision. Longitudinal incisions of the uterus were made in 10 cases because of previous cervico-corporal incisions, transverse lie or placenta praevia.

All patients were observed for 6-17 hours in the postoperative department and all children were taken care of by a paediatrician or by a trained anaesthetist.

Additional surgery was performed in 19 cases (3.5%). Tubal sterilization was carried out in 12 women, unilateral oophorectomies in two cases due to benign cysts, ovarian resections in one woman for the same reason and oophorectomies plus hysterectomy in one patient because of ovarian cancer. Appendicitis was diagnosed in three women and a C S was made at the same time as the appendectomy.

COMPARISON BETWEEN ELECTIVE AND EMERGENCY C S

Out of 539 Caesareans 241 were elective and 298 were made under emergency conditions. The C S was made earlier than the 38th gestational week in

Table III Ten patients with major maternal complications after delivery by Caesarean Section under emergency condition at Huddinge University Hospital October 1972-June 1976

amniotitis + peritonitis
amniotitis + peritonitis + paralytic ileus Abscess of the Douglas pouch which was incised Wound infection
amniotitis Relaparotomy due to abscess in the uterine wall + ileus followed by sepsis The patient was nursed in an intensive care unit for 19 days
relaparotomy due to suspected intraabdominal haemorrhage
paralytic ileus Relaparotomy + secondary wound revision
sepsis
peritonitis + ileus Relaparotomy Stenosis of trachea post op Secondary sut of wound
intraabdominal haemorrhage Relaparotomy followed by sepsis and serious wound infection
uterine atonia + parametritis followed by septicaemia
Five of the patients with serious complications underwent laparotomies

than the 42nd week in 92 of the patients (1%)
both intra operative and post operative complications were encountered The post operative complications have been divided into major and minor ones

Intra-operative complications The number of intra-operative complications was equal in both elective and emergency C S Three bladder injuries were encountered and were duly sutured as was subcutaneous haemorrhage

Post operative complications Major post operative complications were only noted in emergency Caesareans (Table III) In five of the 10 patients with such complications re laparotomies had to be made Minor post operative adversities occurred in 2.5% in the elective C S and in 13.1% in the C S done under emergency conditions The most common complication was fever

There was one maternal death due to severe toxemia corresponding to a maternal mortality rate of 1 per thousand of all abdominal deliveries and 0.1 thousand of all deliveries during this period of time The patient died five weeks after a C S from pulmonary and cerebral abscesses and renal failure There were ten neonatal deaths among the infants delivered by emergency C S corresponding to a neonatal mortality rate of 3.3% Seven of the infants died of severe asphyxia and one of lethal congenital abnormalities Half of the 10 neonatal deaths

were in the gestational age of 29-30 weeks and one in the 43rd week

Two of the infants delivered by elective surgery died during the neonatal period corresponding to a neonatal mortality of 0.8% The two mothers of the infants suffered from serious pre-eclampsia One weighing only 800 g was delivered in the 34th gestational week and died after a few hours from IRDS The other baby was delivered in the 39th week a boy weighing 1300 g and died from Potter's syndrome and a tentorium rupture

The registered disorders in the early neonatal period of the infants delivered by emergency C S was 10.9% compared with a rate of 1.7% in the elective C S group All children delivered by elective surgery and suffering from disorders in the neonatal period were at term In contrast 57.6% of the infants with neonatal morbidity in the emergency group were in the 31st-37th gestational week

The neonatal mortality rate for all C S was 2.2% and the total neonatal morbidity rate was 6.6% There was neither any morbidity nor any mortality of infants delivered by elective C S from healthy mothers at term

DISCUSSION

A threefold increase of C S (3.5-9.7%) has been observed at Huddinge University Hospital during the period October 1972-June 1976 This coincides with reports from several large obstetrical units in USA and Europe where a C S rate of 10-13% has been registered in the 1970s (4-6) A somewhat lesser rate however would be expected in the Scandinavian countries where a primary C S for example is not an indication for repeat Caesareans

A remarkably high frequency of C S in patients aged less than 20 and more than 35 has been noted by Plötho & Podesser in Austria in 1975 (8) This

Table IV Fetal distress (asphyxia) as the main indication for C S October 1972-June 1976 in 103 patients (19.1% of all C S performed) at Huddinge University Hospital

	1972				1976
Asphyxia	3 months	1973	1974	1975	3 months
n	2	10	17	39	35

has not been our experience but might be due to many pregnant women in these age groups demanding abortion in Sweden.

In the present study both the maternal and the neonatal morbidity rate of emergency C.S. exceeded that of elective section by 6.5 times. Moreover the neonatal mortality rate was 4.1 times higher in the emergency than in the elective C.S. group. Thus the elective C.S. can be regarded as a fairly safe procedure both for mother and child.

Fetal asphyxia as an indication for C.S. has become more common during the period investigated (Table IV). In four years 103 cases of fetal distress were noted. In 27 pregnancies (26.2% of all asphyxias) a combination of several prognostically severe indices were observed (low or falling estriol values, intrauterine growth retardation as measured by ultrasound, toxemia). In these high risk pregnancies spontaneous or induced labour by oxytocin resulted in CTG abnormalities which aroused clinical concern and the patients were delivered by emergency C.S. Babies born to such mothers (5% of all C.S. in our study) are known to exhibit glycogen depletion with higher neonatal mortality as well as morbidity (2, 9, 10). It is therefore our attitude to consider delivery by elective C.S. when the fetal hazards of vaginal delivery cannot be appraised. This procedure might however still increase the C.S. rate.

In the remaining asphyxia group the diagnosis of fetal hypoxia was based on CTG abnormalities which served as the sole indications for section. It seems reasonable as shown by several authors (4, 7) that a combination of CTG with fetal blood sampling could rule out several cases of suspected fetal depression and thus eventually decrease the

rate of C.S. Such a prospective study is now in progress at Huddinge University Hospital.

REFERENCES

- 1 Aaro L. A. & Saed F. Low incidence caesarean section: 12 year experience. *Obstet Gynecol* 51: 22-23 1976.
- 2 Beard R. W. The effect of fetal blood sampling on caesarean section for fetal distress. *J Obstet Gynecol Br Comm* 75: 1291-1295 1968.
- 3 Borell U. & Fernstrom I. Radiologisk bevakning av fosteret. *Acta Radiol Suppl* 191 1960.
- 4 Edington P. T., Sibanda J. & Beard R. W. Evidence on clinical practice of routine fetal monitoring. *Br Med J* 3: 341-343 1975.
- 5 Hibbard L. T. Changing trends in caesarean section. *Am J Obstet Gynecol* 125: 798-804 1976.
- 6 Johnell H. E., Östberg H. & Wahlstrand T. Increasing caesarean section rate. *Acta Obstet Gynec Scand* 55: 95-100 1976.
- 7 Lehmann W. D., Neumann G. K., Kessler K. F. & Jonath W. D. Operationshäufigkeit und perinatale Sterblichkeit vor und nach Einführung der fetalen Fetusanalyse und der kontinuierlichen Überwachung der fetalen Herzfrequenz. *Geburtshilfe* 76: 247-255 1976.
- 8 Plotho B. & Podesser H. Sectio caesarea: 5 Jahres Studie der Ergebnisse einer mit einer Abteilung für Geburtshilfe und Gynäkologie. *Fortschr Med* 31: 1533-1536 1974.
- 9 Shelley H. J. The metabolic response of the fetus to hypoxia. *J Obstet Gynecol Br Comm* 75: 111-114 1968.
- 10 Thalme B., Belfrage P. & Raabe N. Duplex Doppler fetal hypoxia medelst skalpblood sampling. *Opusc Med* 17: 66-70 1977.

Submitted for publication Feb. 21 1977

Eva Patek
Dept of Obstetrics and Gynecology
Huddinge Hospital
S-141 86 Huddinge
Sweden

AN INTRAVENOUS ^{133}Xe METHOD FOR MEASURING REGIONAL DISTRIBUTION OF PLACENTAL BLOOD FLOWJyrki Kuikka Kalevi Kaar Pentti Jouppila Tapani Pyöralä
and Ahti Rekonen*From the Department of Clinical Chemistry and the Department of Obstetrics and Gynecology
University Central Hospital Oulu and from the Department of Obstetrics
and Gynecology and the Department of Radiotherapy Central
Finland Central Hospital Jyväskylä Finland*

Abstract A new method for measuring regional distribution of placental blood flow is presented. After 2-5 mCi of ^{133}Xe saline has been injected intravenously the counts over the placenta are registered with a scintillation camera interfaced with a digital data storage and television read-out system. Using "area of interest" technique time activity curves are obtained for different regions. Analysis of these curves is based on two-exponential curve fitting. In normal pregnancy the mean intervillous flow is 16 healthy women was 125 ml/min/100 ml and myometrial flow 17 ml/min/100 g. The intervillous flow in the central part of the placenta was 55% higher than in the marginal parts. The myometrial flow in the placental area was nearly the same as outside the placenta.

A scintillation camera interfaced with a digital computer has been used for measuring the regional distribution of blood flow in various organs such as brain, lungs and myocardium. As yet no way has been presented for evaluating the regional distribution of fetal circulation, though this would be of interest in clinical obstetrics, especially in cases of possible placental insufficiency.

Recently Rekonen and co-workers (5) presented an intravenous ^{133}Xe method for quantitative measurement of placental blood flow. The technique is non-invasive and uses single scintillation detectors. After i.v. injection of ^{133}Xe saline the tracer is distributed in the organism in the same proportion as the blood in the systemic circulation. With a single detector one can only measure the circulation in a particular organ area. But by using a scintillation camera with area of interest capabilities it is possible to evaluate the regional distribution of

scintillation camera interfaced with data storage and a fast television read-out system. The intervillous flow distribution in normal pregnancy is presented. Simultaneously the regional myometrial flow is measured in the area of the placenta and outside this.

MATERIAL AND METHODS

The test subjects were 16 healthy women with uncomplicated pregnancy, mean age 26 years (range 18-37 years). The measurements were performed during the 35th-40th weeks of pregnancy.

Before the measurements the patients refrained from smoking and rested on the measuring table for at least ten minutes. The placenta was localized beforehand by an ultrasound B scan. The patient lay in a 15° left lateral tilted position during the examination. The gamma camera head with low energy collimator (Radica) was aligned on the placental area. A dose of 2-5 mCi of ^{133}Xe saline (gamma radiation energy 80 keV, half life 5.3 d) was injected rapidly into the cubital vein through a two-way syringe and flushed immediately with 10 ml of physiological saline. To diminish the escape of ^{133}Xe through the lungs the patient held her breath for 15-20 s after the injection. To record quantitative data from the placenta a data storage (PDP 8 II & words) and read-out system with fast television techniques (Nukab) was connected to the scintillation camera. The data in digital form were gathered using 64×64 image matrices with a 10 s channel width over 10 min. After the measurements the data were transferred to the magnetic disc. The areas of interest were marked on the placental image for quantitative analysis. The analysis areas were whole placenta, three placental subregions and myometrial tissue outside the placenta (Fig. 1). The total data density over the placenta ranged from 200 to 2000 counts per cm. The peak count rate was 1000-10000 counts/10 s.

CALCULATIONS

The time activity curve of each area was fed in off-line mode into a central computer (NOVA 840).

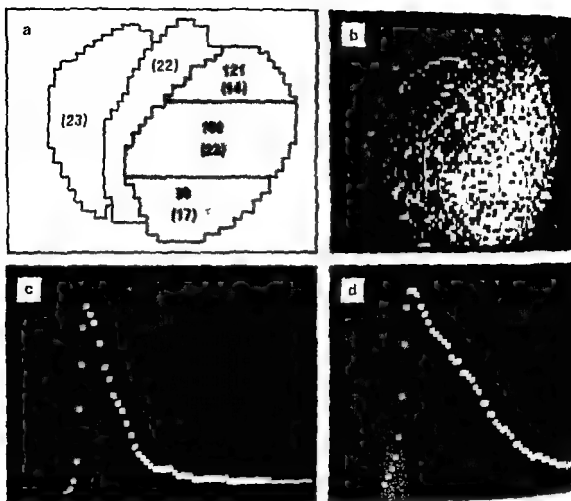


Fig 1 The regional distribution of blood flow in the placenta and myometrium. The mean flow in the intervillous space is 141 ml/min/100 ml and in the myometrial area 19 ml/min/100 g. (a) Distribution of flow: the shaded area is the placenta. Figures without brackets refer to the in

tervillous flow and with brackets to the myometrial flow. (b) The original gamma picture of the placenta. (c) Curve registered over the whole placental area. (d) Curve registered over the myometrial area outside the placenta.

64 k words) for processing. A two-exponential function ($A_1 \exp(-\lambda_1 t) + A_2 \exp(-\lambda_2 t)$) was fitted to the measured washout curve using the least square method. The fast component describes the fractional removal ($dQ(t)/dt = -\lambda_1 Q(t)$) of the tracer from the intervillous blood pool. The regional intervillous flow (in ml/min/100 ml) was calculated from the equation

$$F_i = 100\lambda_1 \quad (1)$$

and the myometrial flow (in ml/min/100 g) from the slow component by using the conventional washout equation

$$F_m = p/\lambda_2 \quad (2)$$

where p is the partition coefficient between blood and myometrial muscle ($=70 \text{ ml}/100 \text{ g}$ haemoglobin, 45%).

The radiation dose to the foetus with an activity of 5 mCi is less than 2 mrad.

RESULTS

In the whole placental area the mean intervillous flow was 125 ml/min/100 ml with a standard deviation of 45 ml/min/100 ml. The myometrial flow in the placental area was $17 \pm 7 \text{ ml}/\text{min}/100 \text{ g}$ and outside the placenta $16 \pm 7 \text{ ml}/\text{min}/100 \text{ g}$. The difference between myometrial flow values in the placental area is not statistically significant ($p > 0.05$).

Fig 1 shows a typical regional distribution of the intervillous and myometrial flows. In ten cases where the low ^{133}Xe dose (2 mCi) was used the count rate in the small subregions was too low for reliable regional analysis. When the higher dose (5 mCi) was injected this analysis was possible (6 cases). In all but two of these women the flow in the central part of the placenta (155 ml/min/100 ml) was on average 55% higher than in the marginal parts (95 ml/min/100 ml). In the remaining two women where the umbilical cord was situated in the marginal part of the placenta no clear difference was found between the subregions of the placenta.

DISCUSSION

The intervillous flow values presented here are of the same magnitude as measured with other methods (2, 3, 4). Rekonen and co-workers (5) have published results from 37 healthy women using the ^{133}Xe single detector method. For intervillous flow they obtained a value of 135 ml/min/100 ml and for myometrial flow 8 ml/min/100 g. The intervillous flow is nearly the same as presented here but the myometrial flow is clearly slower. With the scintillation camera method the measuring area (area of interest) is delineated more exactly and the influence of the low circulation in extramyometrial tissues is smaller than in single detector measurements. The myometrial flow in the placental area did not differ significantly (4% higher) from the flow outside the placenta.

This is in agreement with Rekonen et al (5) and conflicts with the results of Jansson (4) who found a significantly higher flow in the placental area.

The picture of the regional distribution of the blood flow shows that the circulation in the central part of the placenta is substantially higher (55%) than in the marginal parts. This is understandable because in these cases the junction of the umbilical cord was situated in the area of maximal flow. As the distribution of blood flow is clearly non-uniform the detector measurements may vary substantially. This uncertainty can be avoided by choosing a large measurement area with the aid of gamma cameras of the placenta (the area of maximal intensity).

When the measurements of blood flow distribution were made with the same activity (2 mCi) as in single detector examinations the subregional analysis was not successful. But although the activity

has to be 5 mCi or more the radiation load is insignificant on both the mother and the foetus.

The exact analysis presupposes that the tracer input into the intervillous space is an impulse bolus. The i.v. injection actually leads to a broadening and recirculation of this bolus but these distortions are so slight that their influence on the results is insignificant (under 10%) and exact deconvolution analysis is not necessary.

The i.v. ^{133}Xe method for measuring the regional distribution of placental blood flow is atraumatic; it is easy for the patient and the laboratory but does require a sophisticated scintillation camera computer apparatus. The procedure takes 15–20 min of the patient's time and the analysis lasts about 20 min when the placenta is divided into subregions. The method yields a large amount of quantitative information: the situation and form of the placenta, the intervillous flow and its spatial distribution and the myometrial flow. The method is not suitable for studying a placenta situated in the posterior wall of the uterus. In this paper the method has been used in normal pregnancy only. Obviously it is also suitable for examining the distribution of placental blood flow in pathological situations.

REFERENCES

- Clavero J A, Negueruela J, Ortiz L, de los Heros J A & Medrego S P. Blood flow in the intervillous space and fetal blood flow. *Am J Obstet Gynecol* 116: 340, 1973.
- Dixon H G, Browne J C M & Davey D A. Chorionodecidual and myometrial blood flow. *Lancet* 2: 369, 1963.
- Forssman L. Methods of calculating uterine blood flow from the wash-out curves of intra-arterial and local injections of ^{133}Xe . *Acta Obstet Gynecol Scand* 54: 479, 1975.
- Jansson J. Peripheral and myometrial circulation in pregnancy. *Acta Obstet Gynecol Scand* 48: Suppl. 8, 1969.
- Rekonen A, Luotola H, Pitkanen M, Kuikka J & Pyorala T. Measurement of intervillous and myometrial flow by an intravenous ^{133}Xe method. *Br J Obstet Gynecol* 83: 723, 1976.

Submitted for publication March 21, 1977

Jyrki Kuikka
Department of Clinical Chemistry
University Central Hospital
SF-90270 Oulu 22
Finland

ULTRASTRUCTURE OF HUMAN UMBILICAL VEINS

*Observations on veins from newborn children of smoking
and nonsmoking mothers*

Inger Asmussen

*From the Department of Obstetrics and Gynecology YA Rigshospitalet
University of Copenhagen Denmark*

The umbilical vein was chosen as a possible for evaluating the vascular injury provoked by smoking in humans. Umbilical veins from born children of 4 nonsmoking and 4 heavy smoking mothers were examined in the transmission electron microscope. Severe changes were present in the intima-media of veins in the heavy smoker group. Proliferated edema was found in the subintimal space, combined with destruction of intimal elastic membranes, a decreased collagen content and a proliferative action of the myocytes. Similar changes have been found in animal studies involving exposure to carbon dioxide hypoxia or nicotine. The present study supports the concept that tobacco-smoking is harmful to the human vascular system and illustrates the mechanism through which the vascular injury is provoked in heavy smokers. The study also indicates that tobacco-smoking causes severe damage during pregnancy to the cord. Summarized changes might be expected in the newborn children of smoking mothers. Tobacco-smoking should therefore be abandoned during pregnancy. Smoking during pregnancy carries a high risk for the fetus, including an increased risk of abortion and stillbirth, as well as death after delivery. It is also well established that the average weight of newborn children of smoking mothers is 300 g less than that of those born to nonsmoking mothers (1, 5, 11, 12, 17). The present study evaluates the structural changes found in the umbilical veins of newborn children of heavy smoking mothers compared to a control group.

PATIENTS

Pregnant women admitted to the Department of Obstetrics and Gynecology at Rigshospitalet were selected for the study. All patients were examined by the same investigator and each patient completed a questionnaire on smoking habits before and during pregnancy. Eight patients took part in this study: four smokers and four nonsmokers. The smokers had been heavily inhaling cigarette smoke even before pregnancy. The nonsmokers had never smoked.

Women suffering from hypertension, diabetes or other diseases and those with Rh negative blood types were excluded from the study. The patients were somatically healthy before and during pregnancy and all laboratory investigations (hemoglobin, blood glucose) including urinary analyses for glucose and protein were normal. Random tests for urinary estriol during pregnancy revealed normal values.

All the children were delivered at term, were mature and no malformations were found. The clinical data are presented in Table I. In all eight cases the duration of labor was <12 hours.

METHODS

Biopsy and preparation for microscopy

Immediately after delivery of the placenta, a soft plastic catheter was placed in the umbilical vein and about 50 ml of cold Ringer's solution was infused from a syringe under light pressure.

Perfusion was continued with 4.5% cold purified glutaraldehyde containing 2% acrolein and buffered to pH 7.4 with 0.2 M phosphate buffer for at least 5 min to prevent contraction during the cutting procedures.

For the transmission electron microscopy the fixed vein was carefully dissected out, cut into small blocks and immediately transferred to the same glutaraldehyde for 1 hour. Postfixation was performed in 1% buffered osmium tetroxide (pH 7.4) for 1 hour at 4°C. Tissue blocks were dehydrated in graded ethanol, cleared in propylene oxide and embedded in Araldite (Durocupan ACN). Sections 0.5–1.0 µm thick were stained with toluidine blue for light microscopy. Ultrathin sections were cut on glass knives with an LKB Ultratome III, mounted on uncoated copper grids and contrasted with magnesium uranyl acetate and lead citrate. These sections were examined and photographed in a Zeiss EM 9S 2 electron microscope.

The present study is an extension of earlier works on the umbilical artery (3) and the placenta (2).

The photographic work and the description of the morphology were completed and classified without knowledge of smoking habits.

Table I *Clinical data from eight children born to four non smoking and four heavy smoking mothers*

Cigarettes/ day	Weight	Sex	Placenta weight	± Days to term
0	3 900	M	600	+ 8
II	3 900	M	950	- 2
0	4 000	F	950	+ 6
II	3 800	M	750	+14
10	3 150	M	630	-21
15	3 350	F	900	+15
20	3 400	F	660	-10
20	3 343	M	750	- 6

RESULTS

Nonsmokers

The umbilical vein presented with an intima with an endothelial lining of continuous type with closed intercellular junctions a sparse media surrounded by adventitia. The endothelial lining was resting on a non continuous basement membrane. Just beneath the endothelial cells an internal elastic membrane or several elastic membranes were found. In one of the nonsmokers mononuclear white cells were found on several sections of the tissue. The endothelial cells were rich in fine filaments in the cytoplasm thus resembling the underlying myocytes with dense bodies. All the usual organelles were present in the endothelial cells. In the media and adventitia a characteristic finding was the huge amount of collagen fibers.

Smokers

In view of the small number of patients only very striking observations have been recorded. First of all edema was found. It was located just beneath the endothelial cells and above the elastic membranes thus protruding the endothelial wall into the vein lumen. But edema of the endothelial cells and the myocytes of media was also found. The elastic membranes were split up by edema and invasion of myocytes. There was a remarkable decrease in the collagen content of the media and adventitia. By evaluating the collagen content and the presence of edema one could tell whether the tissue came from a smoker or a nonsmoker. The basement membrane underlying the endothelial cells appeared normal.

The endothelial lining also appeared normal

although it was thinner than that of the controls and lipid droplets were often found in the myocytes. Especially in the myocytes lots of glycogen had accumulated.

DISCUSSION

Tobacco smoking is a well-established major factor in the development of atherosclerosis as shown in population studies as well as in animal exposure studies using hypoxia carbon monoxide or nicotine (4 6 7 10 13 14 15 16).

Exposure to these agents causes severe changes in the aorta and coronary arteries as well as in the myocardium of experimental animals. As a possible model in which to demonstrate vascular and tissue damage provoked by tobacco-smoking in humans the placenta and the umbilical artery and vein were chosen for this study.

Severe damage was found in all the specimens from the heavy smokers compared with those from the controls (2 3). The morphological changes could be classified as both degenerative and regenerative.

In the umbilical artery (3) as well as in the umbilical vein edema was a characteristic finding. Similar changes have been described in animal exposure studies. Apparently leakage of the endothelial lining is a major component of the vessel damage leading to subendothelial edema. It is also possible that other plasma components may pass the endothelial barrier and give rise to deposits of fibrin or plasma lipids forming the developing atherosclerotic plaque.

Also the well established proliferative reaction of the media myocytes has been demonstrated in the umbilical artery (3) and vein. In the artery invasion of the broad basement membrane was found while in the veins the internal elastic membrane was split up. The placenta from heavy smoking mothers showed similar changes in the vessels but the most remarkable finding was an obviously decreased vascularization (1).

During pregnancy three different products are formed: the child the placenta and the umbilical cord. The above mentioned changes have demonstrated that tobacco-smoking during pregnancy causes severe ultrastructural as well as microscopic changes in the placenta and umbilical cord. Similar changes must be expected in the children as well but the final proof must wait.

ethical reasons. Children born to smoking women are small compared with controls (3-9). This might be due to damage to the child or sequelae to sick placenta.

In animal exposure studies pregnant animals likewise gave birth to smaller offspring besides producing slightly fewer males (5-8). In humans it has been found that in a highly selected group of heavy smoking women about 80% of the children were females, whereas a control group showed the normal distribution of male and female births in the population.

At present these ultrastructural studies of the umbilical vein artery (3) and placenta (2) are the only published data on the effects of tobacco smoking during pregnancy in humans. Further studies are necessary but the present findings strongly suggest that tobacco-smoking during pregnancy is harmful. In the author's opinion pregnant women should be advised to abandon tobacco-smoking.

ACKNOWLEDGEMENTS

I wish to thank Birte Kargaard for her generous and valuable technical assistance.

REFERENCES

- 1 Andrews J & McGarry J M. A community study of smoking in pregnancy. *Br J Obstet Gynaecol* 79: 1057 1977.
- 2 Asmussen I. Ultrastructure of the human placenta at term. Observations on placentas from newborn children of smoking and non smoking mothers. *Acta Obstet Gynecol Scand* 56: 119 1977.
- 3 Asmussen I & Kjeldsen K. Intimal ultrastructure of human umbilical arteries. Observations on arteries from newborn children of smoking and nonsmoking mothers. *Circ Res* 36: 579 1975.
- 4 Astrup P. Some physiological and pathological effects of moderate carbon monoxide exposure. *Br J Med* 1: 447 1972.

- 5 Astrup P, Trolle D, Olsen H M & Kjeldsen K. Effect of moderate carbon monoxide exposure on fetal development. *Lancet* 1: 1720 1972.
- 6 Constantinescu M & Robinson M. Ultrastructural injury of arterial endothelium. I. Effects of pH, osmolality, anoxia and temperature. *Arch Pathol* 88: 99 1969.
- 7 Constantinescu M & Robinson M. Ultrastructural injury of arterial endothelium. II. Effects of vasoactive amines. *Arch Pathol* 88: 106 1969.
- 8 Essenberg J M, Schwind J V & Patras A. The effects of nicotine and cigarette smoke on pregnant female albino rats and their offsprings. *J Lab Clin Med* 25: 708 1940.
- 9 Fraumeni J F & Lundin F E. Smoking and pregnancy. *Lancet* 1: 173 1964.
- 10 Kjeldsen K, Astrup P & Wanstrup J. Ultrastructural intimal changes in the rabbit aorta after a moderate carbon monoxide exposure. *Atherosclerosis* 16: 67 1972.
- 11 Kullander S & Kallen B. A prospective study of smoking and pregnancy. *Acta Obstet Gynecol Scand* 50: 83 1971.
- 12 Meyer M B & Comstock G W. Maternal cigarette smoking and perinatal mortality. *Am J Epidemiol* 96: 1 1972.
- 13 Shimamoto T. New concept on atherogenesis and treatment of atherosclerotic disease. *Jap Heart J* 13: 537 1972.
- 14 Thomsen H K. Carbon monoxide induced atherosclerosis in primates. *Atherosclerosis* 20: 733 1974.
- 15 Ts'ao C H. Graded endothelial injury of the rabbit aorta. *Arch Pathol* 90: 277 1970.
- 16 Ts'ao C H & Glagov S. Basal endothelial attachment. *Lab Invest* 23: 510 1970.
- 17 Zachau-Christiansen M. The Influence of Prenatal and Perinatal Factors on Development during the First Year of Life. Thesis. Helsingør Denmark 1977.

Submitted for publication March 22 1977

Inger Asmussen
Dept. Ultrastructural Research
Univ. Inst. Pathol. Anat.
University of Copenhagen
Frederik V's Vej 11
DK 2100 København Ø
Denmark

CONJOINED TWINNING IN SWEDEN

Bengt Kallen and Goran Rybo

From the Department of Embryology University of Lund and the Department of Obstetrics and Gynecology Central Hospital Skövde Sweden

Abstract A small epidemic of conjoined twinning is reported occurring at the Skövde Central hospital in 1975-76. Three pairs were observed the annual birth number is 2300. During a ten year period 10 cases of conjoined twinning were reported to the Register of Congenital Malformations in Sweden indicating a probable incidence of 1/75 000 births. These 10 cases were distributed all over the country. Interviews with the three women who gave birth to the Skövde conjoined twins revealed no common factor which could be of aetiological importance.

Recently Bhattay et al (2) reported an apparent increase in conjoined twinning in southern Africa. In 11 cases born during just over 12 months. As assessed by Hanson (3) the actual incidence cannot be evaluated from the figures given and the observed value could agree with the 1/50 000 rate generally thought to be the true rate of conjoined twinning. Earlier Milham (5) gave some evidence of a clustering in time and space of cases of conjoined twinning in the state of New York. He collected 77 cases from the 3.85 million births officially registered during 1945-June 1965 a rate of 1/66 000. This was probably an underestimate of the true incidence. Twelve of the sets of twins were born during 1935-1939 (6 expected) and 6 of these were born in 1939. Five of the latter were born within a region of western New York within 50 miles from each other. The malformation surveillance system in Atlanta (1) found an indication of increasing twinning during 1970-71 no cases of conjoined twinning were found during 1968-69 three each in 1970 and 1971 two in 1972. The possible presence of small epidemics of conjoined twinning suggests that exogenous causes may exist. Our interest was excited by the observation of three cases of conjoined twinning during a little more than one year in one hospital Skövde Central Hospital with approx. 2300 annual births. One case

of thoracopagus was born in April 1975 and another in June 1976. During the same period a late legal abortion was performed on a young girl and the 21 weeks old fetus was dicephalic. As births are only registered officially in Sweden after 28 weeks it was not notified as a case of conjoined twinning. It would however have been included in a study similar to that of Milham where the age limit was 20 weeks.

We report here on this small epidemic and the results of a survey of cases of conjoined twinning reported to the Swedish Register of Congenital Malformations.

THE SKÖVDE CASES

The first birth of conjoined twinning in Skövde Central hospital occurred in April 1975—at 34 weeks gestational age. The mother was a 34 years old house wife with three previous normal deliveries. The twins were thoracopagus boys delivered by caesarean section and were stillborn.

The second case was the legal abortion which was performed in September 1975—corresponding to a gestational age of 21 weeks. The woman was an 18 years old school girl with no previous pregnancy. She was delivered after extraamniotic Rivanol installation of a dicephalic male fetus.

The third case was born in June 1976 at a gestational age of 42 weeks approximately a month after the former case. The twins were female thoracopagus. They were delivered by caesarean section and were live born but an attempt at separation failed and the infants succumbed. The mother was a 30 years old house wife who had one earlier normal delivery.

All three women were questioned about exposure to possible teratogenic agents and for family history of malformations or twinning. The only twinning

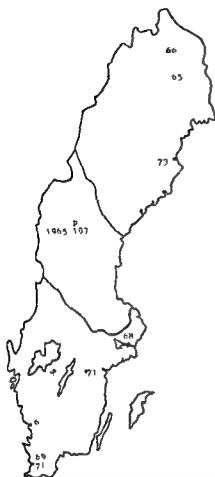


Fig. 1 Map of Sweden with location of cases of conjoined twinning reported to the Register of Congenital Malformations and with the year of birth marked. S marks location of the city of Skövde.

found in the families was dizygotic twins on the husband's side in one of the cases. No similarities were found with respect to factors such as drug use, exposure to chemicals in the environment and radiation.

THE REGISTER OF CONGENITAL MALFORMATIONS

Since 1965 there has been a compulsory reporting system in Sweden: the Register of Congenital Malformations (4). Infants born after 28 weeks gestation are included. A certain underreporting occurs to the register, estimated to be about 20–25% for serious malformations. When an infant is reported, diagnoses are checked and verified. The infants entered under the diagnosis conjoined twinning can be assumed to be correctly described and such in-

fants cannot be entered under other diagnoses such as monsters.

During the 10-years period 1965–1974 10 cases of conjoined twinning were reported. The original patient records of these cases have been scrutinized. The total number of births among hospitals reporting to the register during these years is approximately 878 500 which gives an incidence of about 1/88 000. Only symmetrical conjoined twins are included; not cases of heteropagus. As mentioned, some underreporting may have occurred but it is probably low for such a spectacular malformation as conjoined twinning and an incidence of 1/75 000 in Sweden during this time period seems to be a reasonable estimate.

The births of conjoined twins are fairly well scattered over Sweden (Fig. 1)—the only hospital which had two such births during a ten year period is Malmö hospital (approx. 3500 annual births). The yearly distribution was also even: 7 cases occurred each year in 1965, 1966 and 1971, one case in 1964, 1970 and 1973, none in 1967, 1972 and 1974. The month of the last menstrual period was also even: distributed 2 in January, April and May, none in March and June, one in each of the remaining months. Four pairs were boys, six were girls. Maternal age distribution was wide, from 19 to 45 years. The ten women had together 15 earlier pregnancies. One ended as a legal abortion and three as miscarriages (2 in one woman). Among the 11 infants born, one was a stillbirth.

DISCUSSION

The normal rate of conjoined twinning is uncertain. When routine vital registration is used, estimates are an underestimate; it is probably obtained as cases may be reported without malformation diagnosis or as monsters. The 1/166 000 rate reported by Milham from New York (5) is probably too low. Ascertainment from hospital records, on the other hand, with necessity based on only a few cases. For example, the much quoted figure of 1/50 000 from Potter (6) represents only two cases. It is also probable that a bias is obtained by too high figures as the search of the records is often been made because of the birth of one or more cases of conjoined twinning at one hospital. In the studies reviewed by Rudolph et al. (7) an incidence of 1/31 000 is obtained, which is probably too high. The very varying figures which have

obtained with different methods of ascertainment as well illustrated by Hanson (1975)

We think that a figure of 1/75000 as obtained from the central register can be a fairly adequate estimate of the true incidence in Sweden. This is lower than the figure usually quoted

1/10000. This may be explained partly by the presence of the bias just mentioned as data were collected as part of a routine registration of all malformed infants. The exclusion of cases with a gestational age below 28 weeks may also be a confounding factor and so is the inclusion of only reasonably symmetrical twins. Thus in Milham's series (5) 4 of the 22 pairs were delivered before week 28 and in the Atlanta material one of the nine cases was a parasitic fetus (heteropagus).

If the incidence of 1/75000 is accepted as a reasonable estimate of conjoined twinning incidence in Sweden, the probability of two cases occurring within nine months (calculated from LMP dates) in Skovde with 2300 annual births is very low. The improbability that this has occurred by chance is further strengthened by the phenomenon that within one month before the conception of the second case another conjoined twin pair was conceived although aborted legally before reaching term.

If the three cases occurred as the result of an exogenous influence its nature has not been identified. The women lived at different sites, had different occupations, neither used any drugs nor received infections.

There are two possible ways in which an increase in conjoined twinning can occur. One is an increase in monozygotic twinning with a constant risk for development of conjoined twins, the other is a constant rate of monozygotic twinning but an increased risk of conjoined twinning. It has been stated that an increased incidence of twinning is found in families where a case of conjoined twins is born (8). Rudolph et al. (7) found a positive history of twinning in 13 and a negative one in 6 of 65 reviewed families with conjoined twins. A doubling of the rate of monozygotic twinning would appear as an approximately 30% increase in total twinning rate. The twinning rate in Skovde Central hospital

in 1975 and 1976 was not significantly increased (40 cases found/37 expected).

The data presented in this paper deviate at one point from those usually described for conjoined twinning, namely with respect to sex ratio. It is generally stated that there is a marked predominance of female pairs. For instance of Milham's 22 pairs (5) 20 were female, one was male and one was a mixed male-female pair. Rudolph et al. (7) reviewed 60 cases with known sex—43 were female and 17 male. However, in the relatively recent Atlanta series of 9 cases (1) only 5 were female and in our series of a total of 13 cases, 7 are female. These two series thus give a near 1:1 sex ratio. Is it possible that embryonic loss of male embryos could explain the high female dominance in earlier series? Perhaps better prenatal care reduces embryonic loss and that this results in a more even sex ratio in more recent series—and contributes to an increase of the total rate.

REFERENCES

- 1 Atlanta Center for Disease Control. Congenital Malformations Surveillance, November–December 1977, issued February 1978.
- 2 Bheattay E, Nelson M M & Beighton H. Epidemic of conjoined twins in southern Africa? *Lancet* *ii* 741 1975.
- 3 Hanson J W. Incidence of conjoined twinning. *Lancet* *ii* 1257 1975.
- 4 Källén H & Winberg J. A Swedish register of congenital malformations. Experience with continuous registration during 2 years with special reference to multiple malformations. *Pediatrics* *41* 765 1968.
- 5 Milham S Jr. Symmetrical conjoined twins: an analysis of the birth records of twenty-two sets. *J Pediatr* *69* 643 1966.
- 6 Potter E L. *Pathology of the Fetus and Infant*, p 217. Yearbook Medical Publishers Inc, Chicago 1961.
- 7 Rudolph A J, Michaels J P & Nichols B L. Obstetric management of conjoined twins. *Birth Defects Original Article Series* *3* 78 1967.

Submitted for publication April 4 1977

Bengt Källén
Department of Embryology
University of Lund
Lund
Sweden

ON RECEPTORS FOR ESTROGENS (E_2) AND ANDROGENS (DHT) IN HUMAN ENDOMETRIAL CARCINOMA AND OVARIAN TUMOURS

L G Friberg ■ Kullander J P Persyn and C H Korsten

*From the Gynecological Section Department of Oncology University Hospital Lund
Department of Gynaecology Malmö General Hospital University of Lund
Sweden and the Department of Clinical Chemistry Antoni van Leeuwenhoek
Ziekenhuis Amsterdam the Netherlands*

Abstract Some human endometrial carcinomas contain receptors for estrogen (E_2) and dihydrotestosterone (DHT). Analysis of 10 primary endometrial carcinomas by agar gel electrophoresis revealed a varying receptor pattern: absence of both receptors, sole presence of E_2 or DHT receptor or presence of both. Highly differentiated tumours often had receptors of both types (7/18) and poorly differentiated tumours most often lacked them (1/10). Primary tumours with metastases (Stages III-IV) seldom had combined receptors: only in 2 cases out of 9. Growth of the tumour down in the cervical canal (Stage II) implied absence of combined receptors in all of the 13 cases studied. In 8 different ovarian tumours presence of either E_2 or DHT receptors was found in 6. The findings are discussed in relation to hormonal treatment of the disease.

It is now generally accepted that endocrine therapy can induce regression of certain malignant tumours. The effect of progesterone in the treatment of carcinoma of the uterine corpus has attracted much attention. Particularly well differentiated endometrial carcinomas seem to respond favourably while less and undifferentiated neoplasms seem to be rather clinically resistant. The best effects have been observed on pulmonary metastases in patients over 60 years (8) but also in local recurrences regression in more than half of a series of 37 patients as obtained using only gestagen treatment (1). It seems possible that oestrogens also play a role for the responders (18)—maybe by stimulating target cells to synthesize receptor proteins for binding the progesterone.

In mammary carcinomas it has been shown that endocrine therapy is of little value in the treatment of patients without demonstrable estrogen receptors in the tumour tissue (2, 3, 7, 9, 14 and others). Estrogen receptors have also been found in the en-

dometrium of postmenopausal women. In 8 patients with endometrial carcinomas it was found to bind oestradiol specifically. Specific binding of progesterone in the uterus also seems to exist (14, 17) but whether endometrial carcinoma is hormone-dependent only in those cases where evidence of specific progesterone binding occurs in the malignant tissue is unknown. Hitherto only small numbers of patients with endometrial carcinoma have been evaluated and mostly only for a single receptor: oestradiol. Knowledge of the receptor pattern for several steroids in a greater number of endometrial carcinomas might give an improved picture which would help to refine hormonal therapy. This paper is concerned with the occurrence of oestradiol and dihydrotestosterone receptors in a large group of endometrial carcinomas, the receptors being related to various parameters of the disease. A study in breast cancer has shown evidence that determination of androgen receptors besides the oestrogen receptors would increase the likelihood of predicting clinical responsiveness to castration (3, 15).

Some different ovarian tumours were investigated in this study in the same way as the endometrial carcinomas. So far as is known, no receptor determinations on human ovarian tumours have been published earlier.

MATERIAL AND METHODS

Tumour material was obtained from patients who had not received previous irradiation or hormones. Fifty patients with adenocarcinoma of the endometrium and eight with ovarian tumours were studied. The diagnosis was based on histopathological criteria. The stage of the endometrial cancers was classified according to the International Classification established in 1961. The tissue was collected by

Table I Endometrial cancers

No (n=50)	Patient age	Clinical Stage	Tumour grade of differentiation	
10	≤60 years	I	High	6
			Medium	2
			Low	2
6		II	High	2
			Medium	4
			Low	0
4		III-IV	High	1
			Medium	3
			Low	0
18	>60 years	I	High	7
			Medium	9
			Low	2
7		II	High	2
			Medium	2
			Low	3
5		III-IV	High	0
			Medium	2
			Low	3

curettage or at laparotomy. Small pieces of the tumour was transferred to glass tubes deep frozen and stored until the receptor analysis. A piece of the same tissue was retained for histological evaluation.

Extracts of the tumour tissues were prepared as recommended at the EORTC workshop of September 19 1972 using a microdismembrator (Braun Melsungen Germany). Estrogen and androgen receptor were determined in the extract according to Wagner (21) using agar gel electrophoresis. Incubation with the labelled hormone was done for about 16 hours. The binding of estrogen ($[6,7-^3H]$ estradiol 17β 40 Ci/mole) and androgen (5α dihydro $[1,2-^3H]$ testosterone 48 Ci/mole) expressed as cpm per g tissue protein (counting efficiency 45%). Total protein was assayed using the biuret action. Human serum albumin was assayed immunologically by single diffusion using Partigen plates manufactured by Behring Werke (Germany).

In a previous paper a statistical evaluation was given of the oestrogen binding capacities of benign and malignant human mammary tumours by probit analysis (9). For the discrimination of androgen receptors in breast tumours the same procedure was followed. Intermediate estrogen receptor or androgen receptor values were regarded as positive. The above cut off levels were also applied in the study of oestrogen and androgen binding capacities of endometrial and ovarian tumours.

Clinical details of the endometrial cancer patients and the histopathology of their tumours are shown in Table I.

RESULTS

The results of the receptor determinations in endometrial cancers are shown in Table II and on ovarian tumours in Table III.

Table II Receptors in endometrial cancer

Pat	Stage	No receptors	E _r DHT receptors	Solely E _r	S
Highly differentiated					
≤60 years	I	1	5	0	1
	II	1	0	1	1
	III-IV	1	0	0	1
>60 years	I	2	2	2	1
	II	0	0	2	1
	III-IV	0	0	0	1
Total		5	7	4	7
Medium differentiated					
≤60 years	I	1	0	1	1
	II	1	0	3	1
	III-IV	2	0	1	1
>60 years	I	5	1	1	1
	II	1	1	1	1
	III-IV	1	1	0	1
Total		11	2	7	7
Non differentiated					
≤60 years	I	1	0	1	1
	II	0	0	0	1
	III-IV	0	0	0	1
>60 years	I	2	0	1	1
	II	2	0	0	1
	III-IV*	2	1	0	1
Total		7	1	2	7

* One of these patients G H had skeletal metastases the time of the receptor analysis. Progesterone treatment (400 mg Proluton Depot® 3 times weekly) was started. In spite of this the metastases progressed. Pulmonary metastases developed and the patient died 3 months later.

* One of these patients S A had pulmonary metastases at curettage. Progesterone treatment (400 mg Proluton Depot® 3 times a week) was started immediately. Therapy was without any effect and the patient died 2 months later.

DISCUSSION

Our receptor findings confirm that there is reason to believe that certain ovarian carcinomas respond to endocrine therapy. Gestagens have been observed to be beneficial in cases of pulmonary metastases from endometrial carcinoma (10) and there is clinical evidence that progestagens may be more or less effective in 10% of ovarian cancers. Receptor measurement of a large number of different ovarian tumours is of importance if followed by prospective investigations to find out whether the presence of receptors

Table III Receptors of ovarian tumours

Type of tumour	E_2 receptors	DHT receptors
Mucinous cystadenoma I A	—	+
Mucinous cystadenoma II B	—	+
Cystadenofibroma I B	+	—
Cystadenofibroma I B	—	—
Cystadenocarcinoma II C	—	—
Mucinous cystocarcinoma II C	—	+
Papillary ovarian cancer	—	+
Papillary ovarian cancer	+	—

ovarian cancers really has a value in predicting response to endocrine treatment

Bonte (1) found that the metabolic and hormonal status of the patient with endometrial carcinoma is most significant for the response of the tumour to progesterone therapy. The good responders had along other things an oestrogenic vaginal smear and complete regression was only obtained in postmenopausal patients whose initial estrogenic vaginal smear showed atrophy following gestagen treatment. Obviously those ideal patients had endogenous oestrogens and both oestrogen and progesterone receptors in their vaginal mucosa—and probably also in their endometrium. Influences from sex hormones in postmenopausal women have been found to run parallel in vagina and uterus by many authors using different substances (11).

In our material growth of the endometrial cancer within the cervix canal (Stage II) usually meant absence of combined receptors or DHT receptors. DHT is an oestrogen antagonist (anti-estrogen) and may like progestagens be useful in the treatment of neoplastic changes of the endometrium. In our material tumours with metastases (> Stage II) had relatively seldom combined hormonal receptors. This may explain the failures of endocrine treatment.

Since the presence of sex steroid receptors in a tissue is indicative of its being a target tissue receptor measurements may offer a means for selecting patients with endometrial carcinoma stages III and IV for endocrine therapy.

Two cases mentioned in detail in Table II and lacking receptors were both treated with progesterone but soon died. No other cases of ours were treated with progestagens.

No information is found in the literature on the receptor content of primary endometrial cancers and various secondary tumour deposits analysed at the same time. In different metastases in the same patient there might be different receptor situations due to different cell clones as has been shown for human ovarian tumours in work with other cytostatic agents (12). If no receptors are found in the primary lesion there is little prospect that receptor function is regained in metastatic cell deposits or that hormonal treatment will be successful. On the other hand if receptors are found in the uterine tumour tissue this does not necessarily mean that all or any metastases possess the same receptors.

When demonstrating the occurrence of oestrogen hormone receptors an error might lie in the possibility that in these cases abnormal receptors may occur. The receptor molecules of the tumour cells bind the hormone but may fail to translocate to the nucleus (13). Such false positive cases with abnormal receptors could not be expected to respond.

Radiotherapy may destroy some receptor proteins and thereby also lessen the chances for a hormonal response or influence on the local tumour.

The estrogen receptor concentration of human normal endometrium has been stated (5) to be highest in the early proliferative phase of the menstrual cycle and in the postmenopausal state to be similar to that seen in the proliferative endometrium. In 8 studied cases of endometrial carcinoma according to Evans et al (5) there seemed to be a correlation between the stage of differentiation of the tumour and the receptor concentration being highest in the well differentiated tumours. This was in agreement with results in 9 cases attained by Terenius et al (20) but contradicted findings of Taylor et al (19). Our findings showed that highly differentiated tumours certainly have more often E_2 receptors (12 cases out of 18) than non-differentiated (2 out of 10). They also more often possess receptors of both E_2 and DHT types.

In 15 cases of endometrial carcinoma studied by Follow et al (16) the highest concentrations of E_2 receptors were found in undifferentiated carcinoma which on the other hand showed the lowest concentration of progesterone binding. Negative findings

Table I Endometrial cancers

No (n=50)	Patient age	Clinical Stage	Tumour grade of differentiation	
10	≤60 years	I	High Medium Low	6 2 2
6		II	High Medium Low	2 4 0
4		III-IV	High Medium Low	1 3 0
10	>60 years	I	High Medium Low	7 9 2
7		II	High Medium Low	2 2 3
5		III-IV	High Medium Low	0 2 3

curettage or at laparotomy. Small pieces of the tumour was transferred to glass tubes deep frozen and stored until the receptor analysis. A piece of the same tissue was retained for histological evaluation.

Extracts of the tumour tissues were prepared as recommended at the EORTC workshop of September 19 1972 using a microdismembrator* (Braun Melsungen Germany). Estrogen and androgen receptor were determined in the extract according to Wagner (21) using agar gel electrophoresis. Incubation with the labelled hormone was done for about 16 hours. The binding of estrogen ($[6.7 \text{ }^3\text{H}]$ estradiol 17β 40 Ci/mmol) and androgen (5α -dihydro $[1.2 \text{ }^3\text{H}]$ testosterone 48 Ci/mmol) was expressed as cpm per μ g tissue protein (counting efficiency 45%). Total protein was assayed using the biuret action. Human serum albumin was assayed immunologically by single diffusion using Parthen plates manufactured by Behring Werke (Germany).

In a previous paper a statistical evaluation was given of the oestrogen binding capacities of benign and malignant human mammary tumours by probit analysis (9). For the discrimination of androgen receptors in breast tumours the same procedure was followed. Intermediate estrogen receptor or androgen receptor values were regarded as positive. The above cut off levels were also applied in the study of oestrogen and androgen binding capacities of endometrial and ovarian tumours.

Clinical details of the endometrial cancer patients and the histopathology of their tumours are shown in Table I.

RESULTS

The results of the receptor determinations in endometrial cancers are shown in Table II and on ovarian tumours in Table III.

Table II Receptors in endometrial cancer

Pat	Stage	No receptors	E ₂ DHT receptors	Solely E ₂	Solely DHT
<i>Highly differentiated</i>					
≤60 years	I	1	5	0	0
	II	1	0	1	0
	III-IV	1	0	0	0
>60 years	I	2	2	2	1
	II	0	0	2	0
	III-IV	0	0	0	0
Total		5	7	5	1
<i>Medium differentiated</i>					
≤60 years	I	1	0	1	0
	II	1	0	3	0
	III-IV	2	0	1	0
>60 years	I	5	1	1	2
	II	1	0	1	0
	III-IV	1	1	0	0
Total		11	2	7	2
<i>Non differentiated</i>					
≤60 years	I	1	0	1	0
	II	0	0	0	0
	III-IV	0	0	0	0
>60 years	I	2	0	0	0
	II	2	0	0	1
	III-IV	2	1	0	0
Total		7	1	1	1

One of these patients G H had skeletal metastases at the time of the receptor analysis. Progesterone treatment (500 mg Proluton Depot® 3 times weekly) was then started. In spite of this the metastases progressed. No pulmonary metastases developed and the patient died after 3 months.

One of these patients S A had pulmonary metastases at curettage. Progesterone treatment (500 mg Proluton Depot® 3 times a week) was started immediately. No therapy was without any effect and the patient died after 2 months later.

DISCUSSION

Our receptor findings confirm that there is reason to believe that certain ovarian carcinomas may respond to endocrine therapy. Gestagen treatment has been observed to be beneficial in cases with pulmonary metastases from endometrial carcinoma (10) and there is clinical evidence that progestagens may be more or less effective in about 10% of ovarian cancers. Receptor measurements on a large number of different ovarian tumours are of importance if followed by prospective studies in order to find out whether the presence of receptors

PREGNANCY AND SARCOMA

Kianoosh Jafari Abraham F Lash and Augusta Webster

From the Department of Obstetrics and Gynecology of the Cook County Hospital and the Department of Obstetrics and Gynecology of the University of Health Sciences/The Chicago Medical School Chicago Illinois

Abstract Six cases of pregnancy associated with sarcoma presented. Among these six cases is one patient with ovarian sarcoma which was diagnosed during the 34th week pregnancy. The literature was reviewed with reference to the effect which pregnancy may have on the growth of malignant tumors. Most authors believe that pregnancy has no deleterious effect on the course of cancer, however, this is not well supported through adequate data. Several reports to the contrary contain evidence that pregnancy may have a deleterious effect on the course of cancer. These discrepancies could be explained on the basis of differences in the natural history and biological behavior of different malignant tumors in relation to the pregnancy.

The association of pregnancy with sarcoma is relatively rare. The management of a patient with this condition includes the question as to whether or not pregnancy may have adverse effects on the growth of the tumor. The treatment depends on the site of lesion, anatomic location, stage of the disease and age of the gestation (1, 2).

In the recent management of a patient with metastatic liposarcoma prompted us to review our case files which contain 5 other cases of pregnancy complicated by sarcoma. Table 1 shows a brief summary of our patients with sarcoma and pregnancy who were treated at Cook County Hospital.

In this paper we are presenting a review of the literature and our 6 cases.

CASE REPORTS

Case 1 (C H)

A 35-year-old white female gravida 7 para 1 was admitted with complaint of labor pains. She was pregnant 36 weeks and had dyspnea, orthopnea and marked edema of lower extremities. Fetal heart tones were normal. The cervix was thick and closed. A chest X ray revealed multiple round densities in the right lung field suggestive of pulmonary metastases. Because of heart failure the

patient was placed on nasal oxygen and digoxin. A week after admission her general condition deteriorated progressively and under local anesthesia a classical Cesarean section was performed. A live term female infant was delivered. Evisceration occurred on the eighth postoperative day and was repaired. Subsequently she became febrile and expired on the eleventh postpartum day (4). Postmortem examination revealed a 10x8x6 cm mass located in the posterior and medial aspect of the right leg. Histological examination revealed pleomorphic sarcoma. There were multiple metastases to both lungs.

Case 2 (R H)

This 37-year-old female gravida 11 para 10 was admitted to Cook County Hospital in March 1953 with a history of abdominal pain of two days duration. She was 7 months pregnant and had been bedridden for four months following a fall at home.

Physical examination revealed an emaciated female with a limp right arm and atrophy of the right leg. Fetal heart tones were normal. Bone X rays revealed generalized osteoporosis and sclerosis of the skull. Blood chemistry revealed elevation of serum calcium and alkaline phosphatase. Eight days after admission she went into labor and delivered a 2210-gram infant in good condition. X rays of the infant revealed normal bone structures.

A bone marrow biopsy of the patient revealed metastatic tumor cells. The patient expired on the 89th postpartum day (5). Autopsy revealed a benign parathyroid adenoma and an osteogenic sarcoma.

Case 3 (S P)

This 36-year-old white female gravida 4 para 2 abortus 1 was admitted to the hospital on August 5, 1960 because of jaundice, chills and fever of one week's duration. She had a history of lymphosarcoma which was treated with X ray in 1955. A diagnosis of pulmonary tuberculosis had been made in 1957. She did well until 1958 when the lymphosarcoma became symptomatic again for which she received another course of X ray therapy.

Physical examination on admission revealed a severely icteric, debilitated female. She had a blood pressure of 170/80 mmHg, pulse rate of 140 per minute. There was a systolic murmur at the apex of the heart, rales and ronchi at the base of the left lung. She had hepatosplenomegaly and the uterus was enlarged to 37 weeks gestational size. Fetal heart tones were normal.



Figs 1 and 2 Gross and microscopic examination of vulvar tumor in case 4 (Z.J.) showing malignant mixed mesodermal tumor compatible with sarcoma botryoides



The patient was treated with intravenous fluids, antibiotics, steroids and anti-tuberculosis drugs. After some improvement she went into labor on the 4th hospital day and delivered a 1390-gram live infant. Despite intensive medical treatment the general condition of the patient deteriorated and she expired on the 11th postpartum day.

Case 4 (Z.J.)

This 39-year-old female, gravida 15 para 4, was admitted at 34 weeks gestation to Cook County Hospital with a complaint of a painful mass in the vulvar region. She first noted this as a small mass ten months prior to her admission. It subsequently increased in size during the pregnancy and became symptomatic a few days prior to admission. She was known to have diabetes mellitus since 1958 and was controlled on 45 units of NPH insulin daily during pregnancy.

Physical examination revealed a 264-pound female who was not in acute distress. She had a blood pressure of 140/90 mmHg, a pulse rate of 120 per minute and a normal temperature. Heart sounds were normal and the lungs were clear. Her uterus was enlarged to a 36 weeks gestational size. The fetal heart tones were normal. There was a fist size mass arising from the left side of the vulva obstructing the introitus. Rectal examination revealed a floating head and closed cervix. Her blood glucose ranged from 86 to 174 mg per 100 ml and the chest X-ray was normal. The VDRL test was negative and the hemogram showed hemoglobin of 10.8 g.

Suspecting a Bartholin gland abscess, incision and drainage were attempted but no fluid was obtained. A biopsy was performed and reported as myosarcoma.

One week after admission the patient went into labor. A Cesarean section was performed and a female child delivered. The post partum course was uneventful.

The patient was given 3700 rads external radiation to the perineum which decreased the size of the tumor. Subsequently this was followed by local wide excision of the tumor 2 months post partum. Pathological examination of the specimen revealed a mass measuring 7.0 x 6.5 x 1.5 cm and weighing 98 g. The bulk of the tumor consisted of firm, pale yellow tissue. Microscopic examination of the tumor revealed a mixed mesodermal tumor compatible with sarcoma botryoides (Figs 1 and 2). The patient was lost to follow up since 1967.

Case 5 (M.C.)

This 22-year-old Negro female, gravida 3 para 1, was admitted to Cook County Hospital in August 1969. She was 38 weeks pregnant and gave a history of a previous Cesarean section. She had a giant cell tumor of the left femur treated in 1969 by wide excision of the tumor.

Physical examination revealed a 38 weeks gestational size uterus and normal fetal heart tones. There was a surgical scar on the lower aspect of the left leg. One week after admission the patient underwent a Cesarean section and tubal ligation. A 2450-gram live male child was born. She was discharged on the eighth postpartum day. At present the patient is living with no evidence of disease.

Case 6 (T.N.)

A 15-year-old black female was admitted to Cook County Hospital on September 21, 1974 in the third trimester of pregnancy.



Fig. 3. Chest X ray of case 6 (T₁N₁) showing opacification of the left hemithorax due to metastatic liposarcoma.

pregnancy complaining of right sided chest pain of four weeks duration and productive cough with yellow sputum. She had a history of anaplastic sarcoma of the right thigh treated by 2000 rads external radiation followed by a 5-day course of Actinomycin D (500 microgram per kg per day) and partial resection of the right thigh muscles on the third day of chemotherapy in November 1973. She did well and remained asymptomatic until November 1974. She was then lost to follow up. On admission she had shortness of breath requiring oxygen therapy by mask. Her heart sounds were normal, pulse rate was 100 per minute and her blood pressure 130/80 mmHg. She had decreased breath sounds over the right hemithorax and dullness on percussion of the right posterior lung field. Her uterus was enlarged to a 32-week gestation. Fetal heart tones were normal. The abdomen was soft and admitted one finger tip. There was an old surgical scar on the right thigh. The hemoglobin was 8 g, white blood cell count was 10,000, her liver function and blood chemistry were normal, the coagulation time was normal, urinalysis and urine culture were unremarkable.

The chest X ray showed opacification of the entire right hemithorax (Fig. 3). The trachea was slightly shifted to the left side. The findings were consistent with collapse of the right lung thought to be due to occlusion to the right main bronchus. Superior vena cavagram revealed a filling defect on the right side of superior mediastinum causing acute compression of the distal portion of the superior vena cava and both innominate veins. Esophagogram

showed narrowing of the middle portion of the esophagus suggestive of extrinsic pressure. Ultrasound examination of the abdomen revealed a fetal biparietal diameter consistent with a 31-32 week gestation. Cytology of bronchial washing was reported as Class II. A bronchoscopic biopsy specimen was reported as poorly differentiated liposarcoma metastasizing to bronchial tissue.

On October 6, 1976, the patient was started on external radiation to the mediastinum with shielding of the abdomen. Ten days later she went into labor which lasted for four hours and delivered a 1815-gram male infant with an Apgar score of 10. The postpartum course was uneventful and radiation treatment was completed on October 30, 1976, with a total dose of 3900 rads to the mediastinum. This was followed by a 7-day course of multiple drug chemotherapy consisting of Adriamycin 20 mg intravenously, three doses; Cytosar 400 mg intravenously, two doses; and 5-(3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) 300 mg intravenously, two doses. The patient was heparinized with 5000 units every 4-6 hours during the course of chemotherapy. After the first course of chemotherapy, the general condition improved markedly, she no longer required oxygen, her voice improved and the dysphagia subsided. Because of severe bone marrow suppression, the second course of chemotherapy was started four weeks later with reduced doses and continued with 2-3 week intervals due to the patient's condition. Sequential X rays (Figs. 4 and 5) showed improvement of the involved lung. At present, the patient is in remission and her general condition is good.



DISCUSSION

existence of genital sarcoma and pregnancy is an extremely rare condition. A case of endometrial sarcoma causing obstructed labor was reported by (6) in which the infant was delivered by cesarean section. In 1972 Kececioğlu (7) reported use of sarcoma botryoides of the cervix coincident with first trimester of pregnancy. The patient was treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy followed by 6000 r external irradiation. Sarcoma botryoides arising from the vagina coincident with pregnancy has not been reported (8).

Sarcoma of the vulva is a very uncommon lesion. Investigators noted that this lesion is more likely to occur in patients between the ages of 30 to 40 but it has been reported in a 4-month old infant as well as in a newborn (9). From the late 19th century to the present, as nearly as we are able to determine, only 4 cases of sarcoma of the vulva associated with pregnancy including our case have been reported (9). In 1957 Nolan (10) reported a case of sarcoma arising from the base of the hymen in a 23 year old pregnant patient. In 1961 Lygij (1) and co-workers reported two cases of pregnancy complicated by Ewing's sarcoma of the pubic bone in one and reticulum cell sarcoma of the perineum in the other. They noted a remarkable acceleration of tumor growth during pregnancy and rapid metastatic spread during the postpartum period. And Probst and co-workers (9) in 1966 reported a case of fibrosarcoma arising from the right labium majoris in the fifth month of pregnancy. The patient was treated by hemivulvectomy without node dissection.

It is of importance to the clinician to know whether or not pregnancy has any adverse effect on the growth of the malignant tumor.

Our analysis of the available data does not suggest that pregnancy has any deleterious effect on the course of sarcoma. Treves & Holleb (11) compared groups of pregnant and non pregnant women with breast cancer and found a higher incidence of axillary nodes in a group of pregnant pa-

tients. White and co-workers (12) reported a 73.3% five year survival in a group of 30 patients with melanoma and pregnancy versus 54% five year survival in a group of 31 non pregnant women. They concluded therefore that pregnancy does not have adverse effects on survival of patients with melanoma. George and co-workers (13) reviewed 115 cases of melanoma associated with pregnancy and 330 controls from Memorial Hospital. They demonstrated that there is a higher incidence of regional node metastasis in pregnancy but the clinical course of melanoma in both groups was the same.

Shev and co-workers (14) recently reported a series of 251 patients with melanoma associated with pregnancy. One hundred and sixty five had Stage I disease and 86 patients had Stage II (regional node metastases). In Stage I the overall survival rate was 84% with no significant difference between the two groups however in Stage II the survival rate was significantly lower in pregnant patients than in nulliparous patients 29% and 55% respectively.

Cantin and co-workers (15) reported a series of 205 premenopausal women 57 of whom became pregnant during the course of management of sarcoma. These 57 patients had a total of 111 pregnancies which occurred either simultaneously or subsequent to the treatment of sarcoma or after the appearance of the tumor but before initiation of therapy. They compared the 89% five year survival rate in a group of nine patients with liposarcoma associated with 13 pregnancies with a 66% five year survival in a group of 29 non pregnant patients of childbearing age. They concluded that there was no adverse effect of pregnancy on the survival rate of women with liposarcoma. They mentioned similar observations among other types of soft tissue sarcomas. Of great interest is one of their patients who had a slow growing tumor on the thigh noted at age 34. At age 39 she became pregnant and the tumor grew larger therefore it was excised and pathological examination of the tissue revealed liposarcoma. At age 47 she became pregnant again. The tumor recurred on the thigh and another primary sarcoma appeared on her neck. Most authors believe that pregnancy does not increase the potentiation of growth and metastatic spread of sarcomas. This evaluation has been made by comparing stages of cancer and survival rates in pregnant and non pregnant patients. Therefore as with

Chest X ray of case 6 (T N) after irradiation for metastatic liposarcoma showing improvement in the involved lung.

Chest X ray of case 6 (T N) after irradiation and chemotherapy for metastatic liposarcoma showing improvement in the involved lung.

Table 1 Summary of cases of sarcoma and pregnancy treated at Cook County Hospital

Case	Year of delivery	Age	Gra	Para	Type and location of sarcoma	Treatment	Fetal outcome	Survival & time of report
1 (C H)	1948	35	2	1	Sarcoma (unspecified) of the right leg with pulmonary metastasis	Digoxin Oxygen Cesarean section	Live female	Expired on 1st postpartum day. Autopsy done
2 (R H)	1953	32	11	10	Osteogenic sarcoma	Spontaneous delivery	Live infant 2210 g	Expired 19 hr after delivery. Autopsy done
3 (S P)	1960	36	4	2	Lymphosarcoma	Spontaneous delivery	Live infant 1390 g	Expired 11 hr after delivery. Autopsy done
4 (Z J)	1961	39	15	4	Malignant mixed mesodermal tumor of vulva compatible with sarcoma botryoides	Cesarean section External irradiation (3700 rads) followed by wide local excision of the tumor	Live female infant	Lost to follow-up after 3 months
5 (M C)	1972	22	2	1	Giant cell tumor left leg treated in 1969	Cesarean section and tubal ligation	7450 g live male infant	No evidence of disease
6 (T N)	1975	15	1	0	Liposarcoma of thigh treated in 1973 Metastasis to the lungs appeared during pregnancy	External radiation to mediastinum Vaginal delivery Chemotherapy	1815 g live male infant	In remission

any other retrospective studies it is difficult to find a control group which could properly match the study group. One may argue that a patient with sarcoma who becomes pregnant is very likely in better physical condition than the one who does not become pregnant.

Sholket and Fortner studied transplantation of melanoma in pregnant and non pregnant hamsters and found that pregnancy did not stimulate either the development or the metastatic spread of the disease (13).

The report of Shiv and his colleagues revealed a lower survival rate in Stage II melanoma associated with pregnancy compared with non pregnant women. Nevertheless individual case reports from other series reveal progression or recurrence of the disease during pregnancy suggesting the possibility of a deleterious effect of pregnancy on the course of sarcoma.

As is shown in Table I in our series all pregnancies terminated to the delivery of viable infants. Therefore when the disease is localized appropriate treatment should be instituted but when the disease is widely spread and there is not much hope for survival the fetus should be given

the primary consideration. Fetal or placental metastasis is a rare condition. Of the 19 reported maternal cancer metastasizing to the placenta, 14 have been malignant melanoma. Extension of chorionic villi is associated with poor fetal outcome (3).

REFERENCES

- 1 Lysyj A & Bergquist J R. Pregnancy terminated by sarcoma. Report of two cases. *Obstet Gynecol* 21: 506 1963
- 2 Boronow R C. Extrapelvic malignancy and pregnancy. *Obstet Gynecol Surv* 19: 1 1964
- 3 Sokol R J, Hutchison P, Cowan D & Amelanotic melanoma metastatic to the placenta. *J Obstet Gynecol* 124: 431 1976
- 4 Fitzgerald J E & Webster A. Maternal mortality at the Cook County Hospital. *J Obstet Gynecol* 65: 58 1953
- 5 Webster A. Maternal death at the Cook County Hospital. *Am J Obstet Gynecol* 101: 44 1964
- 6 Nye E. Endometrial sarcoma causing labor. *J Obstet Gynecol Br Comm* 80: 999 1973
- 7 Kececioglu Y. Sarcoma botryoides. *Obstet Gynecol* 39: 77 1972
- 8 Roddick J W Jr & Honig J. Sarcoma of the vagina coincident with pregnancy. *Am J Gynecol* 42: 68 1965

- Phore R III Patton G D & Conti E A Pregnancy complicated by nonpigmented sarcoma of the vulva *Obstet Gynecol* 27 420 1966
- Nolan R P Primary nonpigmented sarcoma of the vulva with report of a case complicating pregnancy *Am J Obstet Gynecol* 73 134 1957
- Treves H & Holleb A I A report of 549 cases of breast cancer in women 35 years of age or younger *Surg Gynecol Obstet* 107 271 1958
- White L P Linden G Breslow L et al Studies on melanoma *J Am Med Assoc* 177 235 1961
- George P A Fortner J G & Pack G T Melanoma with pregnancy report of 115 cases *Cancer* 13 854 1960
- Shu M H Schottenfeld D Maclean B et al

Adverse effect of pregnancy on melanoma a reappraisal *Cancer* 37 181 1976

- III Cantin J & McNeer G P The effect of pregnancy on the clinical course of sarcoma of the soft somatic tissue *Surg Gynecol Obstet* 125 1967

Submitted for publication July 25 1977

K Jafari
Department of Obstetrics and Gynecology
Cook County Hospital
1875 West Harrison Street
Chicago Illinois 60612
USA

CERVICAL ADENO CARCINOMA AND PARTIAL HYDATIDIFORM MOLE

Benjamin Mazor Amicam Bar Am and Liane Deligdisch

Institute of Pathology Ichilov Hospital and Department of Obstetrics and Gynecology A Maternity Hospital Hakuryah Municipal-Governmental Medical Center Sackler School of Medicine Tel Aviv University Tel Aviv Is ael

Abstract Partial hydatidiform mole was found in a 39-year-old grand multiparous Jewish woman having a sigmoid cervical adeno-carcinoma. The patient was treated by surgery followed by internal and external pelvic radiation with excellent results. Four and a half years later the initial diagnosis she is very well. This is the first reported case of a rare combination. The literature regarding the association between pregnancy and cervical malignancies is reviewed briefly and the possible pathogenic relationship between hydatidiform mole and carcinoma of the cervix is discussed.

To the best of our knowledge the simultaneous occurrence of hydatidiform mole and cervical adenocarcinoma has not been reported in the medical literature before.

Krimmenau & Ganjhan (8) in 1960 reported a case of invasive mole occurring in a 45 year-old woman with squamous cell carcinoma of the cervix. The tumor was macroscopically small and had spread to the inguinal lymph nodes.

CASE HISTORY

A 39 year-old woman was first seen in our out patient clinic in April 1977. She was complaining of low abdominal pain, back ache and a whitish gelatinous vaginal discharge for a few weeks. She had seven normal pregnancies and deliveries in the past and one twin delivery 14 years ago. Her periods were every 23 to 30 days. The last one had been on 4th March 1977. On physical examination her general condition was good. No palpable abdominal or pelvic masses were found. Vaginal examination revealed the uterus slightly enlarged with both parametria and the pouch of Douglas free. A soft solid grey coloured mass measuring approximately 3x2x4 cm was protruding from the external cervical os through a pedicle. The mass did not bleed on touch. A white gelatinous vaginal discharge was also noticed.

All routine blood and urine tests were normal. Chest x-ray was negative. Excision of the cervical tumor was performed under general anesthesia without any particular difficulties. Histologically it was found to be a polypoid well differentiated adenocarcinoma of the cervix uteri which consisted of irregularly crowded mucus secreting

glands. The connective tissue between them was scanty. The epithelial cells although most of them well differentiated and mucin producing showed irregularities, nuclear and cellular atypias as well as numerous atypical mitoses. However the general pattern was glandular with a few papillary infoldings (Figs 1 and 2).

Two weeks later a laparotomy was performed. At operation the uterus was the size of an eight week pregnancy. Both appendages were normal. No enlarged lymph nodes were present. Total hysterectomy with bilateral salpingo-oophorectomy was carried out.

At gross examination the uterus measured 11x9x4 cm. The cavity contained a soft reddish mass measuring 7x1x1 cm attached to the fundus with few vesicular structures the diameter of which was up to 0.5 cm. No embryonic structure was found.

Microscopically in the cervix there were found foci of squamous metaplasia and mild dysplasia consisting of irregular stratification and epithelial cells with large hyperchromatic nuclei. The soft mass with vascular structures present in the uterine fundus was a placenta with partial molar transformation. Most of the chorionic villi showed receding vascularity or were avascular with an extensive oedema of the stroma and trophoblastic hyperplasia. Many chorionic villi were extremely enlarged and their stroma was acellular with hydropic degeneration. Sheets of trophoblastic tissue composed of both syncytio- and cytotrophoblasts were found attached to the chorionic villi (Fig 3). No embryonic tissues were found microscopically. Numerous trophoblastic cells were present in the decidual blood vessels. The histopathological diagnosis after hysterectomy was: Pregnant uterus with partial hydatidiform mole. Granulation tissue with squamous metaplasia and mild dysplasia of cervix uteri.

The patient made an uneventful recovery. Two pregnancy tests in high dilutions on the eighth and ninth post-operative days were both negative. After her discharge from hospital she had radiotherapy consisting of 2,000 r of Co⁶⁰ applied to the vaginal vault followed 2 weeks later by 3,800 r applied externally to her pelvis. The second treatment was discontinued because of diarrhoea. The woman remains well and is attending our follow up clinic. All pregnancy tests were negative. A chest X-ray and intravenous pyelography performed in August 1974 were normal. There is no evidence of any metastases. She was last seen in August 1976 when her condition was excellent.

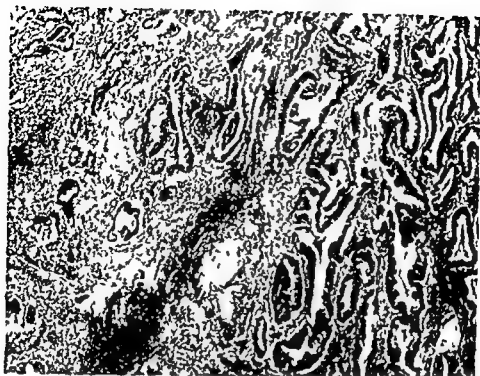


Fig 1 Low power photomicrograph of well differentiated invasive cervical adenocarcinoma. Hematoxylin-eosin stain.

DISCUSSION

Adenocarcinoma of the cervix is found in 5% of all cervical malignancies (2-4). Interestingly enough, while the incidence of carcinoma of the cervix is

generally declining due to cervical cytology, the proportion of adenocarcinoma appears to be increasing (9). Czernobilsky et al (3) found 7 cervical adenocarcinomas in a series of 60 Jewish women.



Fig 2 High power view of cervical adenocarcinoma showing detail of malignant glandular structures. Hematoxylin-eosin stain.



Fig 3 Edematous avascular villi of partial hydatidiform mole
Hematoxylin-eosin $\times 100$

in cervical malignancies. This incidence (11.7%) differs markedly from the general 5% incidence occurring in non Jewish population.

The incidence of carcinoma of cervix in pregnancy varies. Dewhurst (4) stated that one in 5000 one in 20000 pregnancies is complicated by cervical malignancy. Kistner (6) in a review of 136 pregnant women with cervical malignancy noted that about 1% of all cervical cancers occurred in pregnant women and that pregnancy is complicated by this disease once in every 2000 cases. Of all carcinomas found during pregnancy 3% are mucocarcinomas (5, 10). It seems that during the 15 years with the wide and almost routine use of cervical smears the statistical incidence of carcinomas of the cervix in pregnancy has increased (11). Most authors agree that there is no definite evidence that pregnancy has any deleterious effect on the disease during the first and second trimesters (6). Treatment of carcinoma of cervix in pregnancy depends on the stage of pregnancy and degree of malignancy.

The histological diagnosis of carcinoma in situ of cervix in pregnancy is a rather difficult problem because of the various changes in the cervical epithelium which are always present during pregnancy and which can give a false picture of malignancy. The treatment therefore must be very cautious

and rather conservative. If a woman is young and desires more children she can continue with her pregnancy and have the cervix re-examined after confinement (6). Stage I and more advanced cervical cancers found at the beginning of pregnancy necessitate termination of pregnancy to prevent spread of the disease. But even if termination is not performed radiotherapy which is the treatment of choice is usually followed by spontaneous abortion. If the fetus is viable cesarean section is performed followed by radiotherapy. There is a general 39% foetal wastage (11). Vaginal delivery should not be permitted and the abdominal route must always be preferred.

The survival rate in cases treated in the first and second trimesters of pregnancy is almost similar to that of the non pregnant patients but becomes considerably lower if treatment starts after the 32nd week of gestation or early puerperium (5, 7). The importance of early diagnosis is therefore imperative.

We do not know the influence of hydatidiform mole on cervical malignancies as our case is the second reported in the medical literature. We presume that the presence of chorionic gonadotrophin in high levels in the blood circulation as always happens in molar pregnancy has a possible stimulating effect on the cervical tumor.

In our case in a grand multiparous Jewish woman an adenocarcinoma of the cervix uteri was diagnosed and at hysterectomy an unsuspected molar pregnancy was found. The adenocarcinoma was a polypoid mass which at the beginning of its development might have been a glandular polyp. The hormonal influence of the molar pregnancy with its marked trophoblastic hyperplasia could have been the trigger mechanism in the natural history of the transformation of a polyp into an adenocarcinoma although we have no proof that hormonal influences are directly involved in carcinogenesis in the cervix.

The dysplastic proliferation of the cervical epithelium found in the uterus removed two weeks after the complete excision of the adenocarcinoma might also have been influenced by the trophoblastic hyperactivity of the partial mole.

The post operative follow up for 4.5 years has been uneventful and X rays of chest, spine and intravenous pyelography have been normal. We therefore presume that so far metastases can be ruled out.

REFERENCES

- 1 Cramer J K & Hawken S W. Cancer of the cervix and pregnancy. *Obstet Gynecol* 22: 346, 1963.
- 2 Czernobilsky B, Kessler J & Lancet M. Cervical

- adenocarcinoma in a woman on long term contraceptives. *Obstet Gynecol* 43: 517, 1974.
- 3 Czernobilsky B, Rottenstreich L & Lasser Y. Adenocarcinoma of the cervix in Jewish women: a clinico-pathologic study of seven cases. *Isr J Med Sci* 11: 367, 1975.
- 4 Dewhurst C J. *Integrated Obstetrics and Gynecology for Postgraduates* 1st ed. pp 617-67. Eastwell, London, 1972.
- 5 Gustafsson D C & Kottmeier H L. Carcinoma of the cervix associated with pregnancy. *Acta Obstet Gynecol Scand* 41: 1, 1967.
- 6 Kistner R W. *Gynecology Principles and Practice* 2nd ed. pp 164-141. Year Book Medical Publishers, Chicago, 1973.
- 7 Kistner R W, Gorbacek A C & Smith G Y. Cervical cancer in pregnancy. *Obstet Gynecol* 9: 1957.
- 8 Krimmenau R & Ganghanel M. Gleichzeitiges Vorliegen von Mola hydatidosa destruens und Pseudokarzinom. *Zbl Gynakol* 87: 587, 1960.
- 9 Tasker J T & Collins J A. Adenocarcinoma of the uterine cervix. *Am J Obstet Gynecol* 113: 344, 1972.
- 10 Waldrop G M & Palmer J P. Carcinoma of the cervix associated with pregnancy. *Am J Obstet Gynecol* 86: 207, 1963.
- 11 Williams T J & Braek C B. Carcinoma of the cervix in pregnancy. *Cancer* 17: 1486, 1964.

Submitted for publication Nov. 19, 1976

B. Mazor
Department of Obstetrics and Gynecology
Carmel Hospital
Haifa
Israel

OPERATIVE TREATMENT OF RECTAL ENDOMETRIOSIS

S. Rupunen and E. Tarna

*From the Department of Obstetrics and Gynaecology
Turku University Central Hospital Turku Finland*

Abstract 77 patients with rectal endometriosis were operated upon in Turku University Central Hospital in 1976. The main symptom was defecation pain especially during menstruation. Eighteen patients had had previous surgery for endometriosis of the pelvic area. A palpable tumor was found on gynaecological examination in every patient. The tumors did not grow through the rectal wall. Three kinds of operative procedures were used (a simple excision method, a so-called window operation and resection of rectum) and the results were

OPERATIVE TREATMENT

The operative procedures performed are found in Table III. During the operation pelvic endometriosis was found in 24 patients and ovarian endometriosis in 22 patients. The size of the tumors varied from 1×3 cm to 12×3 cm. The tumors were in the sacrouterine level in 15 cases, below the sacrouterine level in 3 cases, above the level in 3 cases and extending from below to above the sacrouterine level in 3 cases. Most often the tumor was located in the anterior wall of the rectum but in 6 cases the tumor extended around the wall to the dorsal side of the rectum.

The literature concerning this subject has so far been scarce. Sampson (3) wrote his first case report 20 years ago but surgical treatment at that time was risky. The biggest series of rectal endometriosis in recent years was published by Gray (6) in 1966 (?). In his article he also discussed surgical treatment. Many textbooks deal with the incidence of this disease and suggest that endometriosis is found in the rectum or the sigmoid colon in about 13% (Kaser, Ikle) of all cases of endometriosis.

OPERATIVE TECHNIQUE

The patients were prepared preoperatively as follows. The bowel was emptied and preoperative Neomycin® was given. The surgical treatment was determined by the findings during the operation and a frozen section was obtained in every case to exclude malignancy. In the excision operation the window that was made in the front wall of rectum was closed end to end in two layers using atraumatic plain catgut 00 in the first layer and plain catgut 0 in the second layer. When endometriosis had extended to the dorsal side of the rectum the simple excision operation could not be performed. In those cases we performed resection of rectum and the length of the resected area varied from 5 to 20 cm. A so-called pull through operation can also be done.

MATERIAL AND METHODS

1969-1976 77 patients with rectal endometriosis were operated surgically in the Women's Clinic of Turku University Central Hospital. Table I shows the age of the patients of whom 11 had had previous surgery for endometriosis. The time between the first operation and the operation for rectal endometriosis was on average 32 months. During the first operation rectal endometriosis was found in 6 cases but in none of these was the rectal endometriosis treated surgically. The patients had the same kinds of symptoms as endometriosis patients generally. The symptoms are shown in Table II. The main complaint of the patient with rectal endometriosis is a change in the bowel function during menstruation. She has either constipation or diarrhea but blood or mucus is not seen in the stool. In the 16 parous patients the average time from the last pregnancy was 10 years. On gynaecological examination a palpable tumor was found in the vaginal space in every patient. A barium enema was performed in 4 cases preoperatively and colonoscopy in 7 patients but the findings were normal.

Table I Age distribution

Age range	No. of patients
21-25	7
26-30	4
31-35	10
36-40	8
41-45	2
46-50	1

Table 2

	Dysmenorrhea	Dyspareunia	Earlier operations for endometriosis
General symptoms	25	14	18
	Diarrhea	Obstipation	Defecation pain
Rectal symptoms	3 (during menstruation)	2	15
	2 (during intermenstrual period)	1	12

Number of patients 27

when rectal endometriosis is growing close to the anus. We have often used this kind of operation in correction rectovaginal fistulas but so far we have not used this operation to treat rectal endometriosis. Endometriosis found in other parts of the pelvic was treated as usual. Bilateral oophorectomy had to be done in 2 cases because of severe ovarian endometriosis. Hysterectomy was performed in 8 cases. The size of the area excised from the rectum was decided by inspection and palpation. At the end of the operation a careful lavage of the pelvis was performed with physiological saline solution and one dose of Hyalostop® (Leo) was instilled into the pelvic cavity.

POSTOPERATIVE TREATMENT

All the patients received antibiotics after the operation either ampicillin intravenously or in the case of allergy cephalothin intravenously. The antibiotic treatment was continued until the first defecation had occurred and at the same time parenteral nutrition was stopped. We used no stimulation for the bowel function. The average time to the first defecation was 4-5 days, the shortest being 3 days and the longest 6 days after the operation.

Postoperative fever occurred in 5 cases (over 38°C). The patients stayed in the hospital 8-14 days, the average being 9-10 days. In every case the histological diagnosis was rectal endometriosis.

FINAL EXAMINATION

All the patients were examined in the hospital 2 months after the operation. None had any rectal

symptoms. The troubles with the bowel function during menstruation had disappeared. There were no strictures of the rectum in the operation. Two cases had barium enemas and the findings were normal.

DISCUSSION

Rectal endometriosis is often associated with deep pelvic endometriosis. In our material rectal endometriosis was found alone in only one case. Usually these patients have already been operated for pelvic endometriosis and when symptoms of rectal endometriosis are sometimes found. A change in the bowel function during menstruation is the main complaint. The patient may have constipation or diarrhea but the stool does not contain blood or mucus because endometriosis does not grow through the rectal wall into the lumen. This is why barium enemas and sigmoidoscopy do not reveal this disease. This is important for the differential diagnosis of carcinoma of the rectum. Nevertheless the finding of a palpable tumor must always suggest the possibility of malignancy. Therefore operation for rectal endometriosis should only be performed in a hospital where there is a facility for a frozen section. This examination should always be done. A frozen section is very useful to confirm the diagnosis of rectal endometriosis.

Surgical treatment of rectal endometriosis should not be attempted if rectal endometriosis is discovered unexpectedly in an unprepared patient. A lateral oophorectomy will of course also lead to regression of rectal endometriosis. On the other hand with careful preoperative management and good surgical technique and postoperative care rectal endometriosis can be cured without doing bilateral ovariectomy at the same time. The operation provides great satisfaction.

Table 3

	Rectal procedures	Hysterectomy
Excision of tumor (without penetrating rectal wall)	7	1
Excision of tumor (window method)	11	4
Resection of rectum	6	-
Pull through	5	-
Bilateral ovariectomy	-	1

patient because it effectively relieves defecation during menstruation. The operator must have experience in intestinal surgery and therefore operation is not a routine gynaecological technique.

REFERENCES

1 Gray L. H. Endometriosis of the bowel: treatment by resection or castration. *South Med J* 58: 815, 1965.

- 2 Gray L. H. The management of endometriosis involving the bowel. *Clin Obst Gyn* 9: 309, 1966.
- 3 Sampson J. A. Intestinal adenomas of endometrial type. *Arch Surg* 5: 217, 1922.

Submitted for publication May 9, 1977

Seppo Ruponen
Department of Obstetrics and Gynecology
University Central Hospital
70520 Turku 52
Finland

TREATMENT OF MENOPAUSAL OESTROGEN DEFICIENCY SYMPTOMS IN HYSTERECTOMISED WOMEN BY MEANS OF 17 β OESTRADIOL PELLET IMPLANTS

B Ståland

From the Samariterhemmet Hospital Gynecological Outpatient Department Uppsala Sweden

Abstract Ninety four women having undergone hysterectomy with or without simultaneous ovariectomy were treated for menopausal symptoms by means of subcutaneous implantation of sterile pellets containing 20 mg β -oestradiol 589 implantations in all. The subjective effect was good and lasted generally for about 6 months. Karyopyknotic index serum FSH and plasma oestradiol were checked in a few of the patients. The method was judged to be valuable in appropriate cases.

β -oestradiol—the most important of oestrogens occurring naturally in man—has long been used for treating menopausal symptoms in the form of esters for injection and in recent years for oral use as esterified and free forms (1 3 4 6 8 10). The use of oestradiol in the form of pellets for subcutaneous implantation is not new. In particular long acting oestradiol esters for injection became available this method was used to a certain extent but only on a small scale. There are only a few references to it in the literature. The author is able to find only one more comprehensive study—that of Muller (9). Chionnda *et al* (2) and Tallworthy (13). Schleyer Saunders (11) and Studd (4) mention the method without giving details of the patient sample. The patients in Muller's investigation were mostly women with intact ovaries. According to present knowledge of oestrogen treatment it is not surprising that bleeding occurred fairly frequently. The method is scarcely suitable for patients with uterus intact. Even with small amounts of oestrogens bleeding readily occurs if the treatment is administered continuously for a long time. Furthermore continuous oestrogen treatment may possibly predispose patients to endometrial cancer. The implantation method should therefore be reserved for women who have undergone hysterectomy. The author has used the

method on such patients for more than 20 years. The preparation used—Dimenformon compr ad implantationem (depot tablets)—was formerly registered in Sweden but over the last 10–15 years has been obtainable for licensed trial by courtesy of N/V Organon. The sample discussed in this paper comprises all patients who have been accepted for treatment during the ten years from 1965 to 1975.

METHOD

The preparation consists of compressed rod shaped pellets containing 20 mg 17 β -oestradiol without excipient. The pellets measure 2 mm in diameter and are 6 mm long. Each pellet is packed sterile in a vial. Their shape makes it easy to implant the pellet subcutaneously using a trocar and cannula. It also means that the surface area and therefore the absorption rate vary little during absorption period.

Implantation is effected with a cannula with the same inside diameter as the diameter of the pellet implant (2 mm) and provided with two trocars—one pointed for introducing the cannula and one blunt for inserting the pellet. The implantations were effected subcutaneously in the lower abdominal wall. The technique is extremely simple. After a little local anaesthetic has been injected intra and subcutaneously a small incision 1–2 mm long is made through the skin with a pointed scalpel blade. Through this the cannula with the pointed trocar is inserted 5–8 cm in the subcutaneous fat nearly parallel to the skin after which the implant pellet is inserted into the cannula and pushed in with the blunt trocar. No suture is required. With a little practice the whole procedure takes less than half a minute. No visible scar is left. Only at one single implantation did bleeding necessitate a suture. Occasionally minor insignificant haematomas have appeared but never any infection or other complication. Any discomfort is insignificant and patients who have also had hormone injections report that the implantations are less unpleasant than injections. When implanting in conjunction with laparotomy the pellet may be inserted from the operation incision with a pair of tweezers into the subcutaneous fatty tissue.

Table I Duration of the effect on climacteric symptoms

Duration (months)	No of implant	% of implant with duration indicated (508)
0-4	2	0.4
4-5	19	3.7
5-6	104	20.5
6-7	254	50.0
7-8	49	9.7
8-	80	15.7
Undefined	81	
Total	589	100

PATIENTS

The series consist of 94 patients, all of whom had undergone hysterectomy—in most cases total—including 46 with simultaneous bilateral ovariectomy. For 15 of the patients the first implantation was effected in conjunction with the operation, for the others from less than 1 year to 29 years afterwards. The ages at the start of treatment ranged from 38 to 72.

The indications for treatment were, in the first instance, typical menopausal disorders in the form of sweating and hot flushes. Less continuous symptoms such as headache, palpitation, muscular pains, insomnia and various mental symptoms often occurred simultaneously. In some older patients the symptoms of sweating and hot flushes were insignificant or completely absent. The indication for treatment was here primarily urogenital symptoms such as vaginal atrophy with symptoms of so-called senile colpitis, dysuria and urgency incontinence, together with difficulties in resuming sexual relations. Even in milder cases of stress incontinence the treatment was of some value.

The series includes altogether 589 implantations. The number of implantations in cases where the effect could be studied was between 2 and 38—for 61 patients at least 5, for 26 patients at least 10 implantations.

RESULTS

The assessment of the effect was based primarily on information given by the patients themselves. The subjective effect was very good throughout. Only 2 patients reported an unsatisfactory effect on sweating and hot flushes. Many patients had previously various forms of oestrogen treatment and practically all preferred the implantation method. The patients also included an appreciable number who had previously been treated with sedatives and similar preparations without any effect whatsoever. It is often stated that the placebo effect when treating menopausal symptoms is high. Figures up to 35% have been indicated (5). This does not tally

with the author's own experience nor with recent investigations. Launtzen (6) indicates a cure of 10-13%. The very poor effect of self itself constitutes evidence against a very high degree of placebo effect.

No direct comparison with placebo was made in the present investigation, though efforts in this direction were made, however, when implantation was effected at the same time as a bilateral ovariectomy, the patients were informed about implantation but were asked, if any climacteric symptoms appeared. The duration of effect for this first implant is the same for the patients as a whole. The mean duration of implantation in conjunction with operation was 5.9 ± 0.03 months. For all cases together 6.1 ± 0.05 months. Difference 0.2 ± 0.3 months.

Presumably the absorption time was curtailed if the pellet was fragmented and this in fact was observed when it was unintentionally broken during implantation. This seemed to be a chance to determine to what extent the idea of the effective duration might be influenced, the check up being usually arranged for 6 months later. In 21 cases selected at random the pellets were deliberately broken into 2-3 pieces before implantation. This was not recorded in the notes, but the doctor should not unintentionally break a pellet when asking about the duration of the effect. The notes concerning fragmentation were not made out until we went through the material for the study. This showed as anticipated that the effect was of value before the disorders returned was indeed longer than usual. It varied between 4 and 6 months with an average of 5.2 ± 0.15 months compared with 6.1 ± 0.05 months for the whole sample. The difference was 0.9 ± 0.16 months and therefore statistically significant.

Table I shows the duration of the subjective effect. As a rule the patients were treated for 6 months. This period was modified in some cases according to the duration of the effect. The patients were requested to note the time of onset of sweating and flushes and were usually given a prescription for some oral oestrogen preparation when necessary. Only in exceptional cases they needed to avail themselves of this. The durations indicated in the table are to be regarded as minimum ones as those patients who had a further implantation had not always a recurrence of symptoms at that time. The

Table II Objective parameters

Parameter	Before treatment			3 months			6 months			Months Difference	
	No.	Variation	Mean	No.	Variation	Mean	No.	Variation	Mean		
FSH	11	0-10	2.0	6	3.5-6.6	4.6	6	10-40	18.5	0-3	44.1
										0-6	16.5
										3-6	-27.6
H	5	6.7-15	10.5 \pm 1.7	10	0.4-9.2	3.7 \pm 1.0	16	0.5-14	4.1 \pm 0.8	0-3	6.8 \pm 2.0
										0-6	6.4 \pm 2.4
										3-6	0.4 \pm 1.3
pmol/l	3	37-97	56.3 \pm 21.8	7	69-330	195.1 \pm 34.1	15	59-247	152.5 \pm 14.7	0-3	138.8 \pm 40.5
										0-6	96.2 \pm 26.3
										3-6	42.6 \pm 37.1

t Test $p < 0.05$ t Test $p < 0.005$

months means at least 6 months but may exceed 7 months. If symptoms had not recurred at two consecutive implantations, the time for check up was postponed by one month. If there were no symptoms even then, the treatment was concluded to be summed when necessary. 0-4 months means that the effect had a duration of less than 4 months and was incomplete. The title undefined indicates cases where it was not possible to judge the effect or the duration more accurately—chiefly patients with virtually only urogenital symptoms. The table shows that in a large majority of cases a completely satisfactory effect was obtained for about half a year. Individual variation was rarely more than 1 month except in cases with fragmentation of the pellet. On the other hand, some patients consistently requested rather more frequent implantations and did others. It is remarkable how accurately the patients could detect the time of return of the symptoms as a rule within one or two weeks. In order to gain a more objective idea of the hormone effect, objective parameters were used for smaller groups of the sample. Thus we studied the karyopyknotic index, serum FSH and plasma oestradiol. Estimation of FSH and oestradiol was generally performed 3 and 6 months after implantation. The results are given in Table II. For 3 patients more detailed studies were carried out in connection with the first implantation. In these cases FSH and oestradiol were determined immediately before implantation and then after 1 week, 2 weeks and 1, 3, 5, 6 and 7 months (Fig. 1). For 6 patients even at the first implantation the karyopyknotic index was determined before implantation and again after 3 and 6 months (Table II).

As shown by Fig. 1 the oestradiol levels rise and the FSH starts to fall within one week, which tallies well with the patients' own statements about the disappearance of the climacteric symptoms. The levels then remain fairly constant for up to about 5 months. By 6 months the levels have regained or begun to approach the original levels.

Plasma oestradiol and serum FSH were determined by radioimmunoassay (15). The upper limit for FSH in women of childbearing age is 3 μ g/l. Postmenopausally the values range from 5 μ g/l upwards.

The karyopyknotic index started to fall at 6 months but in none of the 6 cases studied was it as low at 6 months as at the beginning of treatment.

The FSH and oestradiol values at 6 months had by no means always returned to the original levels. The quickest to react is plasma oestradiol, as is to be expected, since this is the primary parameter. It is also the one which best corresponds to the return of menopausal symptoms. As a rule menopausal symptoms return when the plasma oestradiol falls below 100-120 pmol/l. Serum FSH certainly gives a good idea of the effect of an oestrogen but need not necessarily drop despite a good effect of treatment. This seldom occurs, however, and in fact did not do so for any of the patients studied in the present sample. For one patient, however, the drop in FSH was appreciably less than usual, 15.92 and 14 μ g/l at 0, 3 and 6 months respectively. Despite this the effect was very good and the disorders had not returned after 6 months. On the other hand, the one and only patient whose plasma oestradiol did not reach 100 pmol/l after 3 months was one of the two cases where the effect was unsatisfactory. Unfortun-

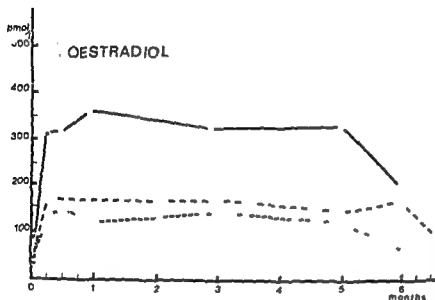
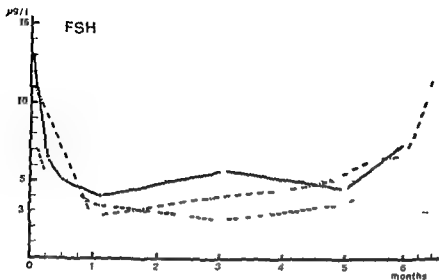


Fig 1 Serum FSH and plasma oestradiol after subcutaneous implantation of 3 mg 17 β -oestradiol at the first implantation (3 cm)

nately oestradiol determination was not carried out for the second of these patients

The appearance of the vaginal epithelium and the vaginal and—when cervix was left—the cervical secretion also showed a picture of strong oestrogenic influence

SIDE EFFECTS

Mastalgia was reported by 4 patients all over the age of 60. Only one of them regarded it as severe enough to want to switch to different treatment. Otherwise no side effects were reported.

Since all patients had undergone hysterectomy

no bleeding could occur. Likewise endometrial cancer does not of course come in on the postmenopausal. There were no cases of mammary or cervical cancer nor of thrombosis. In all the women covered approximately 300 patient years.

Treatment was discontinued for 10 patients: one account of mastalgia (changeover to weak oestrogen treatment) and in 2 due to lack of effect. In one of these the patient was satisfied with oestrogen injections. In the other to high oral dose of oestradiol. Three patients stopped treatment because they felt that they no longer needed oestrogens and 4 changed over on oral treatment on practical grounds.

DISCUSSION

most important of the natural oestrogens in—17 β oestradiol—has proved to be extremely useful for treating menopausal symptoms both at oral and parenteral administration. With oral treatment the doses must, however, be kept considerably higher than for alkylated oestrogens (e.g. ethynylloestradiol). On the other hand, many studies (3, 6, 7, 16 etc.) have demonstrated advantages compared with these, chiefly as regards the effect on blood lipids, osteoporosis and the coagulation system.

The method described here—subcutaneous implantation of 17 β -oestradiol in the form of sterile pellets—is not suitable as a general oestrogen treatment because the absorption time is too long to allow of the requisite breaks in the treatment at 1–2 monthly intervals. However, this does not seem likely to be a drawback for a patient whose uterus has been removed. In such circumstances the very long duration affords considerable advantages. It is usually always possible to achieve a wholly satisfactory effect with two implantations per annum. The method is simple and is preferred by many patients to other methods of administering oestrogens. It is remarkable that such small amounts of oestrogen are required to keep the patients free of symptoms. With an effective duration of 6 months the daily dose of oestradiol can be calculated to approximately 0.1 mg compared with about 0.3 for injections of long acting oestradiol and at least 1 mg with oral administration of oestradiol or oestradiol esters. If the postmenopausal endogenous production of oestradiol is estimated at about 80 μ g/24 h (12) the method would afford a total amount of oestradiol of nearly 1 μ g/24 h which corresponds closely to normal oestrogen production in the years before the menopause (5). The relatively low but constant oestrogen level may be the reason for many patients feeling that they feel better with the implantation treatment than on oestrogens otherwise administered. The effect is nearly always complete whilst on the other hand signs of too high oestrogen level such as mastalgia and oedema occur only in quite exceptional cases.

The disadvantage of course is that the method involves seeing a doctor. However, the time involved in the treatment is very short. The implantation method seems to be particularly valuable in bilateral ovariectomy. We can thereby completely

obviate the often very severe oestrogen deficiency symptoms which are the immediate sequelae of the operation—an added strain in the postoperative condition of stress experienced by the patient.

REFERENCES

- 1 Borglin N E & Staland B Oral treatment of menopausal symptoms with natural oestrogens. *Acta Obstet Gynecol Scand Suppl* 43 1 1975
- 2 Clorinda N, Bohler S & Grenblatt R T The pathophysiology of the hot flush. *The Menopausal syndrome*. Medcom Press New York 1974 p 29
- 3 Furuhyelm M Ostrogenbehandling hos kvinnor efter menopausen. *Läkartidningen* 71 605 1974
- 4 Grenblatt R Therapy for postmenopausal females. *N Engl J Med* 272 305 1965
- 5 Lauritzen C Endocrinology of the menopause and the postmenopausal period. In *Klimakteriet. Endokrine, metaboliske og kliniske aspekter*. Copenhagen 1973
- 6 Lauritzen C The female climacteric syndrome. Significance, problem, treatment. *Acta Obstet Gynecol Scand Suppl* 51 47 1976
- 7 Lebech P E & Borggaard B Changes in serum lipids and anti thrombin III effected by synthetic and natural oestrogen therapy. In *The menopausal syndrome*. Medcom Press New York 1974
- 8 Lebech P E & Svendsen P Oral treatment with oestradiol. *Acta Endocrinol (Kbh) Suppl* 138 1969
- 9 Muller C Die Implantation von Reinsubstanz oestrogenen Hormons bei der Frau. *Schweiz Med Wschr* 83 1127 1953
- 10 Rauramo L, Lagerspetz K, Engblom P & Punnonen R The effect of castration and peroral oestrogen therapy on some physiological functions. *Acta Obstet Gynecol Scand Suppl* 51 3 1976
- 11 Schleyer Saundier E Social and gerontological problems of the menopause. Hormonal implants in *The menopausal syndrome*. Medcom Press New York 1974
- 12 Speroff L, Glass H & Kase N Clinical Gynecologic Endocrinology and Infertility. Baltimore 1974
- 13 Stallworthy J Discussion. *Proc Royal Soc Med* 66 B 1973
- 14 Studd J Management of the menopause. Two views. *Personal view I Prescribers J* 16 51 1976
- 15 Wide L, Nilnius J, Gemzell C & Roos P Radioimmunoassay of follicle stimulating hormone and luteinizing hormone in serum and urine from man and woman. *Acta Endocrinol (Kbh) Suppl* 11 347 1973
- 16 Åstedt B & Jeppson S 17 β -oestradiol and the fibrinolytic activity of vein walls. *J Obstet Gynecol Br Comm* 81 719 1974

Submitted for publication July 29 1977

Bertil Staland
Ostra Ågatan 29
S-753 22 Uppsala
Sweden

SHORT COMMUNICATION

ABSORPTION OF A 8 HYDROXI QUINOLINE (STEROSAN®) THROUGH THE VAGINAL MUCOSA

Olav Meinik Karl Gosta Nygren Christina Fagerlund
and Per Hartvig*From the Department of Obstetrics and Gynecology and the Department of Pharmacy
University Hospital Uppsala Sweden*

chloroquinaldol (5,7-dichloro-2-methyl-8-quinoline) in the form of vaginal jelly (Sterosan®) is presently used as an antibacterial and antimycotic agent in gynecological praxis. The active compound is a compound of the 8-hydroxy-quinoline type, of which have been made responsible for the venous side effect usually known as subacute optic neuropathy (SMON) (6, 7). Recently chloroquinol, another 8-hydroxyquinoline, was found to penetrate the human skin (2). In the present study the absorption of chloroquinaldol through the vaginal mucosa is determined after the application of one dose of vaginal jelly (Sterosan®).

SUBJECTS AND METHODS

Five healthy, regularly menstruating women applied at home a 5 g dose of Sterosan® vaginal jelly (Geigy) containing 1% of chloroquinaldol. Venous blood samples were drawn at 0, 1, 3, 6, 12, 24 and 48 hours. The plasma was separated and stored at -18°C until analysed. Urine was collected in 24 hours samples for three days and stored as described above. The stability of chloroquinaldol on storage has been verified (3). The plasma concentration of chloroquinaldol was determined by electron capture gas chromatography after active methylation (3). Using a plasma volume of 0.5 ml, down to 3 ng/ml could be detected. The urinary excretion was measured using 0.01-0.1 ml aliquots. The amount of chloroquinaldol excreted as conjugated metabolites was determined after hydrolysis with β -glucuronidase at 37°C and pH=4-5 over night.

RESULTS AND DISCUSSION

Plasma concentration of chloroquinaldol

Chloroquinaldol from vaginal jelly (Sterosan®) was found to be readily absorbed from the vagina. One hour after application detectable amounts appeared in plasma. Mean plasma concentrations from the

three women during 24 hours after administration are shown in Fig. 1. Maximum concentration appeared after 12 hours, reaching a mean concentration of 16 ng/ml. After that the concentration slowly declined and on the third day no chloroquinaldol could be detected in any of the three women.

It has been shown that after oral administration of 300 mg of chloroquinaldol a peak plasma concentration of 7 µg/ml appeared after two hours (5). It must be pointed out that chloroquinaldol absorbed from the gut reaches the systemic circulation after partial detoxification in the liver. After vaginal administration, however, the absorbed amount of chloroquinaldol reaches the systemic circulation without being detoxified.

Urinary excretion of chloroquinaldol

Considerable amounts of chloroquinaldol appeared in the urine on the first and second day. In two

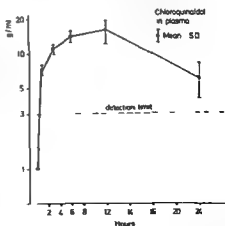


Fig. 1 Mean plasma levels (\pm SD) of chloroquinaldol in three women after application of 5 g of vaginal jelly (Sterosan®).

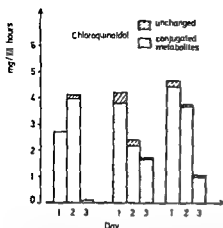


Fig 2 Urinary excretion of unchanged chloroquinaldol and conjugated metabolites in three women. In the first urine specimen from the first woman only the amount of conjugated metabolites was measured

of the women detectable amounts were also found on the third day. More than 90% of absorbed chloroquinaldol was excreted in urine as conjugated metabolites. This is in accordance with findings with other 8-hydroxyquinolines (1-4). The total excretion in urine in the three women amounted to 14, 15 and 18% respectively of the vaginal dose. The daily excretion of unchanged chloroquinaldol and conjugated metabolites is shown in Fig. 2.

CONCLUSION

These results show that so-called local therapy with Sterosan® vaginal jelly results in the absorption of

chloroquinaldol from the vagina into the systemic circulation. This finding must be of importance for the assessment of side effects of this drug, especially for long-term treatment and during pregnancy.

REFERENCES

- Berggren L & Hansson O. Absorption of antiseptics derived from 8-hydroxyquinoline. *Pharmacol Therap* 9: 67, 1968.
- Fischer T & Hartvig P. Slimming with 8-hydroxyquinolines. *Lancet* i: 601, 1970.
- Hartvig P & Fagerlund C. Extraction of biological samples of chloroquinol and chloroquin and determination by electron capture chromatography. *J Chromatography* 141: 179, 1970.
- Haskius W T & Luttermoser H W. Elimination of Vioform and Diodoquin in rabbits. *J Exp Ther* 109: 701, 1953.
- Haegermark B, Wahlberg J E & Gertman. Determination of oxiquinoline concentration in plasma in a patient treated for acrodermatitis atrophicans—an aid in therapeutic control. *matologica* 149: 29, 1974.
- Meade T W. Subacute myelopathy and chloroquinol. *Brit J Prev Soc Med* 29: 174, 1974.
- Oakley G P. The neurotoxicity of 8-hydroxyquinolines. *JAMA* 225: 397, 1973.

Submitted for publication Dec 5, 1977

Olav Meinik
Department of Obstetrics and Gynecology
University Hospital
S-750 14 Uppsala 14
Sweden

CASE REPORT

HYPERVITAMINOSIS A IN EARLY HUMAN PREGNANCY AND MALFORMATIONS OF THE CENTRAL NERVOUS SYSTEM

Lars Stånge Kjell Carlström and Margareta Eriksson

From the Department of Obstetrics and Gynaecology Sabbatsberg Hospital Karolinska Institutet and the Department of Pediatrics St Goran Hospital Karolinska Institutet Stockholm Sweden

act A case of malformations of the fetal central nervous system following hypervitaminosis A in early pregnancy is reported. The mother was treated with 20 IU vitamin A daily during gestation days 19 to 40. Excretion of urinary oestrol carried out in the 4th trimester of pregnancy revealed a very low excretion (4.7-6.6 nmol/24 h). Induced delivery resulted in a microcephalic child who died after 111 min. The child had multiple malformations of the central nervous system and very small adrenal glands (1.5 g normal 11±4 g). The very low urinary oestrol excretion is well explained by the hypoadrenalism which in turn can be related to insufficient ACTH stimulation, a condition similar to anencephaly. Malformations shown in the present case are considered to be related to the high doses of vitamin A given to the mother and the authors wish to warn against uncritical use of high doses of vitamin A in women of childbearing age.

communication describes a case in which the mother was treated with high doses of vitamin A during early pregnancy for this condition. She showed a very low urinary oestrol excretion during later stages of pregnancy and gave birth to a child with malformations of the central nervous system who died shortly after delivery.

METHODS

Urinary oestrol excretion

The excretion of oestrol in the maternal urine was determined by the method of Frandsen (3).

Determination of placental steroid sulphatase activity

Rapid determination of the placental steroid sulphatase was carried out by the following technique. 1.2 g of frozen placental tissue was thawed, washed with 0.06 M Tris-HCl pH 7.2 and homogenized for 60 sec with 5 ml of this medium in an Elvehjem Potter homogenizer at +4. The homogenate was centrifuged at 6000 rpm for 10 min and the supernatant was collected.

The assay mixture consisted of 0.4 ml diluted placental homogenate or 0.4 ml Tris buffer control and 745 cpm [¹⁴C]-dehydroepiandrosterone sulphate (specific activity 550 Ci/mol) in 7 µl of ethanol. The samples were incubated at 37°C for 15 min. All incubations were carried out in duplicate. After that 0.2 ml of 0.5 M sodium phosphate buffer pH 7.5 was added and the liberated [¹⁴C]-dehydroepiandrosterone was extracted with 1 ml of toluene. After centrifugation the toluene layer was taken to liquid scintillation counting of the ¹⁴C radioactivity. Protein concentration in the placental homogenates was determined by the biuret method (9).

CASE REPORT

The mother was aged 26 years, healthy and pregnant for the second time with normal delivery of a healthy child 3 years earlier. In early pregnancy the mother visited a

hypervitaminosis A has been known to be teratogenic in experimental animals since 1953 (1). It was shown to induce congenital anomalies in (2). Since then numerous studies in different animal species have confirmed these results (14). Various types of malformations have been reported, among them anomalies of the central nervous system (1, 15). In humans the teratogenic potential of excessive intake of vitamin A has been discussed (4, 10, 15). However, only two case reports exist so far which suggest a possible relationship between hypervitaminosis A and malformations in humans (1, 12). In the reported cases the infants were born with central abnormalities after maternal intake of 20 and 40 000 units of vitamin A daily during the 3rd trimester. Vitamin A has recently found a new therapeutic indication for treatment of acne (7). The present

Table 1 Urinary oestrol excretion ($\mu\text{mol}/24\text{ h}$) and placental sulphatase activity ($\mu\text{mol liberated dehydroepiandrosterone } \times (\text{mg protein})^{-1} \times (\text{min})^{-1}$)

	Hyper vitaminosis A patient	Placental sulphatase deficiency	Normal control case	Reference values 38th to 42nd week of pregnancy
Urinary oestrol excretion days before delivery				
4		7.9	174	
3	6.6		112	36-198
2	4.2		143	
1		4.8	170	
Delivery at week	42	38	38	-
Placental sulphatase activity	66	3.0	146.0	-

dermatologist because of acne and was prescribed a vitamin A preparation (Ido A®) 50 000 units three times daily. She took this from gestation day 19 to day 40 when she stopped because she realized that she was pregnant. The rest of the pregnancy was normal until the expected delivery day. When the patient was still not delivered 10 days after term, determination of the urinary oestrol excretion was carried out revealing very low excretion values (Table 1).

X-ray examination showed a considerably smaller head than would be expected in relation to the fetal body. The fetus seemed to be microcephalic and therefore delivery was induced by amniotomy and oxytocin infusion. After 5 hours of labour a 3 100 g baby was born that was obviously microcephalic. It died after 18 min.

The autopsy revealed a brain (weight 130 g) that was macerated with considerable amounts of blood in the skull cavity. The ventricles were grossly dilated and the parenchyma of the cerebrum was only 1 cm thick. Between the third and fourth ventricles there was a stricture of the aqueduct. The cerebellum was not pathologically affected. The kidneys were hypoplastic together weighing only 0.5 g. The adrenal glands were small (1.5 g, normal 1.1 ± 0.4 g). The circulatory, respiratory and digestive organs were normal otherwise.

The placenta (530 g) was without pathological changes. In order to exclude a placental steroid sulphatase deficiency as a possible cause to the low maternal urinary oestrol values the placental sulphatase activity was determined. The sulphatase activity was found to be in the same magnitude as in a normal control subject and far higher than in the case of placental sulphatase deficiency (Table 1).

DISCUSSION

The development of the gross morphology of the brain takes place in the 3rd to 5th week of fetal life, i.e. when this mother was treated with high doses of vitamin A. Hypervitaminosis A has been discussed

as a possible pathogenic mechanism for malformations of the central nervous system. Such malformations can be induced in animals by administration of vitamin A (6). It has also been observed that higher postpartum maternal levels of vitamin A were present following the birth of infants with CNS malformations than in the birth of healthy babies (9). In the two reported cases with a possible relationship between intake of vitamin A and birth of a malformation of the urogenital tract was observed (12).

The low maternal urinary oestrol was well explained by the severely hypopituitary condition similar to that found in our patient (4) and which is considered to relate to deficient ACTH stimulation.

It has been reported by Piziak and Cowie (10) that vitamin A greatly increases the amount of progesterone in guinea pig placenta. It has an analogous effect on the corresponding human placental enzymes which might cause an increased conversion of other 20-oxosteroids such as 5-pregnen-20-one-3-sulphate and this in turn might interfere with the transformation of these steroids into oestrogen precursors dehydroepiandrosterone sulphate. It has been reported by Nakamura (11) that excess vitamin A decreases the uptake of inorganic sulphate and the formation of chondroitin sulphate in cartilage. One might speculate over an analogous effect on the sulphurylation of $\Delta^3,3\beta$ hydroxy steroids interfering with the transformation of these steroids into oestrol. However these effects are much less likely. A placental sulphatase deficiency

out by the sulphatase activity figures given in Table 1. The dose ingested by the mothers described in Table 1 were 25 000 and 40 000 units respectively. In the present case the mother took 150 000 units daily during the critical period. The recommended daily allowance for vitamin A during pregnancy is 5000 units, an increase of 25% over the allowance for the nonpregnant adult female (13). Treatment of acne with a dose of 100 000 units daily has been recommended (7). Since acne is a common condition during childbearing age we wanted to report this case with a possible relationship between hypervitaminosis A and later birth of a malformed child to warn against uncritical use of doses of vitamin A in women of childbearing age.

REFERENCES

- Emhardt I B & Dorsey D J Hypervitaminosis A and congenital renal anomalies in a human infant *Obstet Gynecol* 43 750 1974
- Ohlson S G Excessive intake of vitamin A as a cause of congenital anomalies in the rat *Science* 177 535 1973
- Jørgensen V A The Excretion of Oestrol in Normal Human Pregnancy Munksgaard Copenhagen 1963
- Jørgensen V A & Stakemann G The site of production of oestrogenic hormones in human pregnancy *Acta Endocrinol (Kbh)* 38 383 1961
- Sal I, Sharman I M & Pryse Davies J Vitamin A in relation to human congenital malformations *Adv Teratol* 5 143 1972
- Salter H & Warkany J Experimental production of congenital malformations in strains of inbred mice by maternal treatment with hyper vitaminosis A *Am J Pathol* 38 1 1961
- Klingman A M, Mills O H & Leyden J J Acne vulgaris a treatable disease *Postgrad Med* 55 99 1974
- Langman J & Welch G W Excess vitamin A and development of the cerebral cortex *J Comp Neurol* 131 15 1967
- Lowry O H, Rosebrough W J, Farr A L & Randall R J Protein measurements with the Folin phenol reagent *J Biol Chem* 193 265 1951
- Muenter M Hypervitaminosis A *Ann Int Med* 80 105 1974
- Nakamura H Analysis of limb anomalies induced in vitro by vitamin A (retinol) in mice *Teratology* 12 61 1976
- Pilotti G & Scorta A Ipervitaminosi A gravidica e malformazioni neonatali dell apparato urinario *Min Ginec* 87 1103 1965
- Pitkin R M Vitamins and minerals in pregnancy *Chn Perinatol* 2 221 1975
- Pizniak V K & Gawienowski A M The effect of added vitamin A alcohol on the metabolism of progesterone in the placenta of the guinea pig *Cavia porcellus* *Comp Biochem Physiol* 42B 201 1972
- Reuter H & Hellnegel K P Überdosierung von vitamin A *Hippokrates* 45 504 1974
- Shenefelt R E Animal model Treatment of various species with large dose of vitamin A at known stages in pregnancy *Am J Pathol* 66 589 1972

Submitted for publication Jan 14 1977

Lars Stånge
Department of Obstetrics and Gynecology
Sabbatsberg Hospital
11382 Stockholm
Sweden

ANNOUNCEMENTS

During the 6th European Congress of Perinatal Medicine held in Vienna Austria from August 30 to September 1 1978 the *Maternity Prize* of the European Society for Perinatal Medicine will be awarded for outstanding experimental clinical and organizational achievements in the field of Perinatal Medicine. President's address: Prof Dr O Thalhammer Univ Kinderklinik Währinger Gürtel 74 A 1090 Wien Austria

The Second World Federation of Nuclear Medicine and Biology Meeting will be held in Washington D C USA September 17-21 1978. For further information write to Administrative Secretariat Second International Congress of Nuclear Medicine and Biology Suite 700 1629 K Street NW Washington D C 20006 USA

International Society for Cryosurgery. Organized in 1974 the *International society for Cryosurgery* was established to foster the continued development and application of

cryosurgery for the controlled cryogenic destruction of abnormal tissues.

An integral part of this development and application is not only in advances in instrumentation and technique of technique toward attainment of improved clinical palliation and treatment but in the forefront of the biochemical physiological immunological morphological alterations following freezing.

Presently the *Society* holds an International Congress every third year where the latest results in each discipline are presented and discussed. State of the art round tables in which leading authorities address the *Society* has been a major feature at the congresses.

The *Society* welcomes applications for membership from individuals whose qualifications and interests have been demonstrated by experience and publications in relevant field. Applications for membership may be obtained from the *Society's* Secretary Dr Mario Lu Via Nizza 39 10125 Torino Italy. Annual dues are \$100. Successful applicants will be billed following the next membership year.

OVARIAN FOLLICULAR APPARATUS AND HORMONAL PARAMETERS IN PATIENTS WITH PRIMARY AND SECONDARY AMENORRHEA

Ryosuke Nakano Nobuyuki Hashiba Motoo Washio and Shimpei Tojo

From the Department of Obstetrics and Gynecology Kobe University School of Medicine Kobe Japan

Abstract Correlation between ovarian follicular apparatus and hormonal parameters such as serum gonadotropin and urinary estrogen levels was investigated in patients with primary and secondary amenorrhea. In patients with gonadotropin levels were elevated in amenorrheic patients without ovarian follicles or with follicles of low developmental stage and pituitary responsiveness to LH-RH in these patients were marked compared with patients with follicles of high developmental stage or normal ovulating women in the follicular phase of the menstrual cycle. The 24-hour urinary excretion of total estrogens was low in patients without follicles or with follicles of low developmental stage and ovarian responsiveness to exogenous gonadotropins was quite low in comparison with patients with highly developed follicles. Normal control subjects. Thus serum gonadotropin, urinary estrogen measurements and LH-RH and gonadotropin loading tests are diagnostic of the presence or absence and the state of development of ovarian follicles in the diagnosis and treatment of primary and secondary amenorrhea.

In the diagnosis and treatment of amenorrhea, a histologic finding of the ovary, especially knowledge of the presence or absence of ovarian follicular tissue, is of paramount importance and ovarian biopsy either by laparotomy, laparoscopy, or culdoscopy is an accepted procedure for making a histologic assessment. Although efforts have been made by several investigators to associate hormonal parameters such as urinary or plasma gonadotropin levels with the presence or absence of ovarian follicles, correlation has not been sufficiently demonstrated (12-14). In 1973, Goldenberg et al. (6) re-examined the possibility of obtaining the necessary diagnostic information indirectly by correlating the presence or absence of ovarian follicular tissue with measurements of urinary gonadotropins and plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) values in patients with primary and secondary amenorrhea. They reported that a single

plasma FSH value was found sufficient to diagnose the presence or absence of ovarian follicles in every amenorrheic patient.

The present paper is of the correlation between the state of ovarian follicular apparatus and hormonal parameters such as serum LH and FSH levels and the 24-hour urinary excretion of total estrogens in patients with primary and secondary amenorrhea.

MATERIALS AND METHODS

41 amenorrheic patients were studied. Twenty patients had primary amenorrhea and twenty-one patients had secondary amenorrhea. Ovarian biopsy was performed in the diagnostic evaluation of these amenorrheic patients. Specimens were fixed, sectioned and stained with hematoxylin and eosin and evaluated microscopically for absence (Fig. 1) or presence (Figs. 2 and 3) of ovarian follicles. The cases with follicles were further classified into two groups: cases with (1) follicles of low developmental stage (primordial-secondary follicle) (Fig. 2) and (2) follicles of high developmental stage (tertiary-Graafian follicle) (Fig. 3).

The patients were grouped on the basis of primary and/or secondary amenorrhea, absence or presence of follicles, developmental stage of follicles and the etiologic diagnosis of the disorder (Table I).

Eleven normal female volunteers whose ages ranged from 24 to 30 years served as controls. All the women had regular ovulatory menstrual cycles with intervals between periods from 28 to 32 days. The subjects were studied during the follicular phase of the menstrual cycle (cycle day 7). Determinations of the follicular phase of the cycle was based on (1) knowledge of the length of five or more of the most recent consecutive menstrual cycles, (2) daily basal body temperature readings, and (3) the timing of the subsequent menstrual cycle.

Serum LH and FSH levels were measured in duplicate by a double antibody radioimmunoassay utilizing reagents provided by the Human Pituitary Program of the National Institute of Arthritis, Metabolism and Digestive Diseases according to the method of Odell et al. (11) and Midgley (7) with modifications. Both Second International Reference Preparation of Human Menopausal Gonadotropin

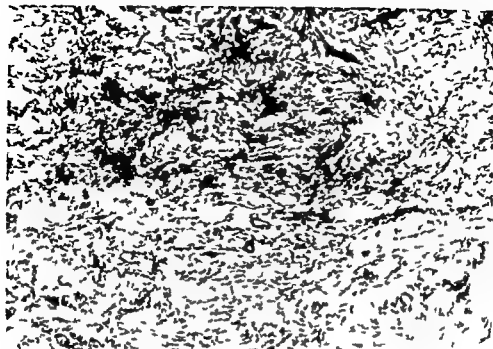


Fig 1 Ovary of an amenorrheic patient without ovarian follicles. Streak ovary with fibrous tissue in the syndrome of gonadal dysgenesis (H&E $\times 70$)

(2nd IRP-HMG) and LER 907 were used to obtain standard curves. The average relative potencies of these preparations were 304 IU of 2nd IRP-HMG/mg LET 907 for LH and 44 IU of 2nd IRP-HMG/mg LER 907 for FSH. The results of this study were expressed as mIU of 2nd

IRP-HMG/ml of serum. Total urinary excretion was measured in 24 hour samples according to the method of Brown et al (2).

Normal control subjects and amenorrheic patients were each subjected to a luteinizing hormone rele-

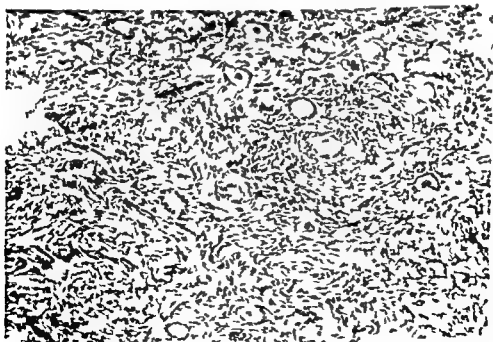


Fig 2 Ovary of an amenorrheic patient with ovarian follicles at an early developmental stage. Numerous un-

stimulated primordial follicles and several antral follicles up to the antral stage (H&E $\times 70$)

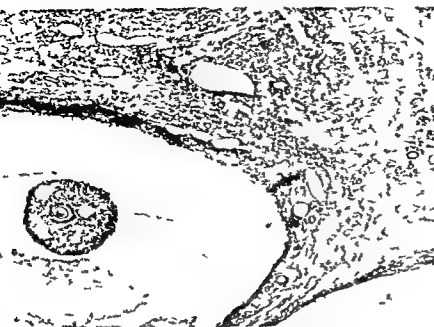


Fig. 3. Ovary of an amenorrheic patient with ovarian follicles at a late developmental stage. Advanced tertiary

follicle surrounded by an active ovarian stroma (H&E $\times 8$)

test (LH-RH) loading test and a gonadotropin load test in order to test pituitary and ovarian response. The synthetic LH-RH used in this study was synthesized and generously supplied by Daichi Seiyaku Co. Tokyo. Blood was sampled at time zero and at 15, 45, 60 and 120 min for determination of serum LH and HCG. A gonadotropin loading test was performed according to the following schedule and the ovarian response was evaluated by daily estimation of the 24 hour urinary

excretion of total estrogens. The human menopausal gonadotropin (HMG) used was Humegon (Organon, Amsterdam, the Netherlands). Each ampule contained 75 IU (2nd IRP-HMG) of FSH activity and 44 IU (2nd IRP-HMG) of LH activity. The human chorionic gonadotropin (HCG) was generously supplied by Teikoku Hormone, Tokyo, and its activity was calibrated against Second International Standard of Human Chorionic Gonadotropin (2nd IS-HCG). HMG (150 IU) and HCG

Table 1. Distribution of patients with primary and secondary amenorrhea with absence or presence of ovarian follicles according to their probable diagnosis

Primary amenorrhea		Secondary amenorrhea	
Absence of follicles		1 Absence of follicle	0
Turner's syndrome	4		
Gonadal dysgenesis with normal karyotype	6		
Presence of follicles		2 Presence of follicles	
Early developmental stage (Primordial-secondary follicle)		(i) Early developmental stage (Primordial-secondary follicle)	
Primary ovarian failure (Atrophic or hypoplastic ovary)	3	Premature menopause	4
Unknown etiology	3	Unknown etiology	1
Late developmental stage (Tertiary-Graafian follicle)		(ii) Late developmental stage (Tertiary-Graafian follicle)	
Primary ovarian failure (Atrophic or hypoplastic ovary)	~	Pituitary Adenoma	
Unknown etiology	2	Sheehan's syndrome	1
		Forbes Albright syndrome	1
		Premature menopause	1
		Polycystic ovary syndrome	5
		Unknown etiology	6
Total	20	Total	21

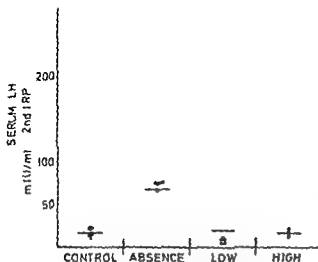
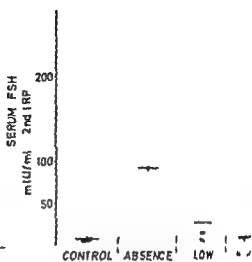


Fig. 4 Serum LH and FSH levels in patients with primary amenorrhea without ovarian follicles (absence) and with follicles at an early (low) or late (high) developmental



stage compared with those in normal ovulating women in the follicular phase (control)

(3000 IU) were administered by intramuscular injection on the 1st and 3rd days of the gonadotropin loading test and total urinary estrogens were measured for five consecutive days.

Informed consent was obtained from each subject after the purpose and nature of the study had been fully explained.

Statistical significance of the data was tested at the 0.05 and 0.01 levels by Student's *t* test.

RESULTS

Serum LH and FSH levels in ten patients with primary amenorrhea without ovarian follicles were higher than those seen in normal ovulating women in the follicular phase of the menstrual cycle ($p < 0.01$). In contrast, serum gonadotropin levels in patients with follicles were similar to those in normal control subjects (Fig. 4).

The twenty-one patients with secondary

amenorrhea had ovarian follicles either at an early developmental stage (primordial to secondary) or at a late developmental stage (tertiary to antral follicle). Serum LH and FSH levels in five patients with follicles at an early developmental stage were elevated compared with normal women in the follicular phase ($p < 0.01$). On the other hand, serum gonadotropin levels in patients with follicles at a late developmental stage did not differ from those in normal control subjects (Fig. 5).

The 24-hour urinary excretion of estrogens in patients with primary amenorrhea without ovarian follicles or with follicles at an early developmental stage was lower than that in normal subjects in the follicular phase ($p < 0.01$ and $p < 0.05$, respectively). Urinary excretion of estrogens in patients with follicles at a late developmental stage did not differ from that in control subjects (Fig. 6).

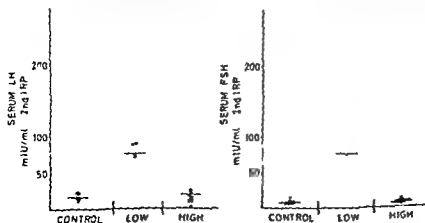


Fig. 6 Serum LH and FSH levels in patients with primary amenorrhea with ovarian follicles of early (low) or late (high) developmental stage compared with those in normal ovulating women in the follicular phase (control).

CONTROL | ABSENCE | LOW | HIGH

The 74-hour urinary excretion of total estrogens in women with primary amenorrhea without ovarian follicles and with follicles at an early (*low*) or late developmental stage compared with that in normal ovulating women in the follicular phase (*control*)

Estrogen levels in four patients with secondary amenorrhea with follicles at an early developmental stage were lower than those in normal ovulating women in the follicular phase ($p < 0.05$). Seven patients with follicles at a late developmental stage showed approximately the same estrogen excretion in urine compared with normal control subjects (Fig 7).

The effect of synthetic LH-RH on serum LH and FSH levels in normal ovulating women in the follicular phase is illustrated in Fig 8. Synthetic

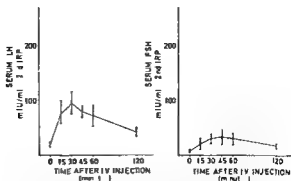


Fig 8 The effect of synthetic LH-RH on serum LH and FSH levels in normal ovulating women in the follicular phase. Bars indicate S.E.

LH-RH stimulated a concomitant release of LH and FSH in these subjects.

Synthetic LH-RH evoked a marked release of LH and FSH in patients with primary amenorrhea without ovarian follicles compared with normal control subjects. In contrast, LH and FSH response to LH-RH in most patients with follicles was approximately analogous to that seen in normal women in the follicular phase. Only one patient with follicles at an early developmental stage showed a marked response to LH-RH (Fig 9).

Serum LH response to LH-RH in patients with secondary amenorrhea with follicles at an early developmental stage was higher than that in control subjects. Although LH response to LH-RH in most patients with follicles at a late developmental stage

CONTROL | LOW | HIGH

The 74-hour urinary excretion of total estrogens in women with secondary amenorrhea with ovarian follicles at an early (*low*) or late (*high*) developmental stage compared with that in normal ovulating women in the follicular phase (*control*)

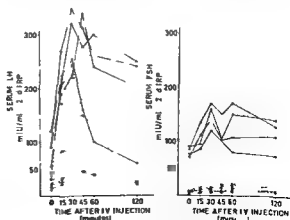


Fig 9 The effect of synthetic LH-RH on serum LH and FSH levels in patients with primary amenorrhea without ovarian follicles (○-○) and with follicles at an early (●-●) or late (●) developmental stage.

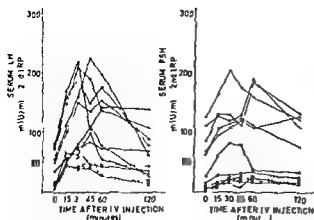


Fig 10 The effect of synthetic LH-RH on serum LH and FSH levels in patients with secondary amenorrhea with ovarian follicles at an early (●—●) or late (○—○) developmental stage

was similar to that seen in normal women. LH response in five patients with polycystic ovary syndrome was extraordinarily high and response in one patient with Sheehan's syndrome was subnormal. Serum FSH response to LH-RH in patients with secondary amenorrhea showed a marked contrast. FSH response in patients with follicles at an early developmental stage was high, but response in patients with follicles at a late developmental stage was similar to that seen in normal women in the follicular phase of the menstrual cycle (Fig 10).

The ovarian response to exogenously administered HMG and HCG on cycle day 5 and 7 in eleven normal ovulating women in the follicular

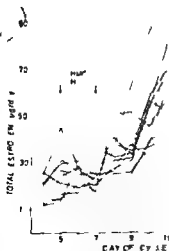


Fig 11 The ovarian response to exogenously administered HMG and HCG on cycle day 5 and 7 in normal ovulating women in the follicular phase

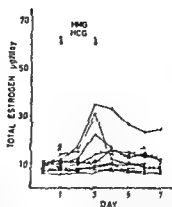


Fig 12 The ovarian response to exogenously administered HMG and HCG on day 1 and 3 of the gonadotropin loading test in patients with primary amenorrhea without ovarian follicles (○—○) and with follicles at an early (●—●) or late (●—●) developmental stage

phase was shown in Fig 11. All the responses corresponded to exogenous gonadotropins with a peak in the 24-hour urinary excretion of total estrogen.

The gonadotropin loading test was carried out in the same manner in thirteen patients with primary amenorrhea and nineteen patients with secondary amenorrhea. No ovarian response was observed in five patients with primary amenorrhea without ovarian follicles. About half of the eight patients with follicles at early or late developmental stage were responsive to HMG and HCG (Fig 12).

Although five patients with secondary amenorrhea with ovarian follicles at an early developmental stage did not respond to HMG and

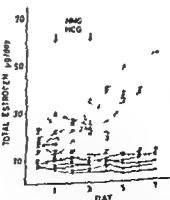


Fig 13 The ovarian response to exogenously administered HMG and HCG on day 1 and 3 of the gonadotropin loading test in patients with primary amenorrhea with ovarian follicles at an early (●—●) or late (●—●) developmental stage

eleven of the fourteen patients with follicles at the late developmental stage responded with a marked increase in urinary excretion of total estrogens (13).

DISCUSSION

Histologic findings of the ovary especially knowledge of the absence or presence of ovarian follicular apparatus and of the state of follicular development is quite important in the diagnosis and treatment of primary and secondary amenorrhea. Knowledge of the ovary for the assessment of amenorrhea has been advocated as part of the clinical investigation (5 15 17 18). Histologic study of ovarian biopsies was made with particular reference to follicular tissue and a correlation was shown between the state of the follicular apparatus and the subsequent clinical progress (20). Provided some follicular element can be seen in the ovary, successful induction of ovulation would be expected. Ovulation would be, however, persistent and unresponsive to stimulant treatment in amenorrheic patients where no follicular tissue can be found on histologic examination.

On the other hand, it is generally accepted that an ovarian biopsy is not always free from hazards. Thus attempts have been made to associate clinical parameters such as serum gonadotropin or steroid hormone levels with the ovarian histology with special reference to the follicular element but correlation has often not been recently demonstrated (12 13 14). In 1973, Tenberg et al. (6) measured total urinary gonadotropins, plasma LH and FSH in normal women and in patients with primary and secondary amenorrhea and respectively correlated with the presence or absence of ovarian follicles. According to their results, total urinary gonadotropin levels

LH values were greater in patients with follicles than in patients without follicles and women but the degree of overlap prohibited diagnosis in any individual patient. Only a FSH value reliably predicted the presence or absence of ovarian follicular element in women with primary or secondary amenorrhea. In this series, serum LH and FSH measurements upon a reliable radioimmunoassay was diagnostic of the presence or absence of follicles in patients with primary amenorrhea and also diagnostic of the state of development of follicles in patients with secondary amenorrhea. The 24 hour

urinary excretion of total estrogens was significantly lower in patients with primary amenorrhea without ovarian follicles or with follicles at an early developmental stage and in patients with secondary amenorrhea with follicles at an early developmental stage. Thus total urinary estrogen values also have diagnostic significance concerning knowledge of the presence or absence and the developmental stage of ovarian follicular apparatus.

Synthetic LH-RH has proved to be an effective means of testing the pituitary secretory reserve for LH and FSH. Pituitary responsiveness to LH-RH in patients with hypogonadism has been reported by several investigators (1 8 9 23 24). It is reported that gonadotropin response to LH-RH in ovarian hypogonadism is significantly higher and response in pituitary hypogonadism is lower than that in the follicular preovulatory and luteal phases of the menstrual cycle. However, little is known about the correlation between the state of follicular growth and the responsiveness to LH-RH. The results of the present study suggest that LH and FSH response to LH-RH is high in amenorrheic patients without ovarian follicles or with follicles at an early developmental stage and moderate or low in patients with follicles at a late developmental stage.

The ovarian responsiveness to exogenously administered gonadotropins has been reported by several investigators (3 4 10 16 21 22). These results indicate that ovarian refractoriness to gonadotropins suggests ovarian failure and ovarian responsiveness suggests hypothalamic ovarian failure. In the present study, the ovaries with follicles at a late developmental stage responded sufficiently to exogenous gonadotropins, whereas most of the ovaries without follicles or with follicles at an early developmental stage did not. The ovarian response to exogenous gonadotropins is considered to be closely correlated with the presence or absence and the developmental stage of follicular element.

Studies on correlation between ovarian morphology and hormonal parameters are of importance in the diagnosis and treatment of primary and secondary amenorrhea and further studies are needed.

ACKNOWLEDGEMENTS

We acknowledge the generous supply of materials for radioimmunoassay of human pituitary gonadotropins by the Human Pituitary Program of the National Institute of Arthritis, Metabolism and Digestive Diseases. The Sec

OVARIAN BIOPSY IN THE EVALUATION OF AMENORRHEA

Karam Karam and Adnan Mroueh

From the Department of Obstetrics and Gynecology American University Hospital Beirut Lebanon

act Endoscopic ovarian biopsies were performed on menorrhagic patients in an attempt to determine the type of their amenorrhea and predict its prognosis relating the histologic examination with physical findings, endocrine patterns and cytogenetic studies. Ovarian lesions were present while gonadotropins were high in 14 cases (6 primary 8 secondary) and there were no follicles in 3 (3 primary 1 secondary) whose gonadotropins were low. Secondary sex characteristics were well defined without prior estrogen stimulation in 5 cases of primary amenorrhea who had no follicles and whose gonadotropins were either low 3 or high 2. The mere presence of ovarian follicles was not enough to make them responsive to gonadotropin stimulation whether endogenous or exogenous a phenomenon that had to do with the type and quantity of germinal follicles available. The histologic examination of ovarian tissue for the evaluation of amenorrhea has been made feasible and relatively safe through recent advances in endoscopic techniques.

amenorrhea is a symptom of wide variety of disorders whose evaluation and treatment largely depend on a clear knowledge of ovarian function. For assessment we generally rely on indirect methods like physical examination, gynecography, and assay of gonadotropin output and chromosomal studies. Though helpful yet these indices may not reflect the exact status of the ovaries. Knowledge of ovarian histology although very informative has not been utilized fully because of the morbidity and expense incurred in laparotomies or ovarian biopsies. Laparotomy was considered only in a disparity between clinical findings and laboratory investigation existed (14).

Recently there has been a resurgence of interest in both diagnostic and operative endoscopy. This has been due to advances in technology, instrumentation and refinements in culdoscopic and laparoscopic techniques. Operative procedures have been made through the culdoscope as early as 1963 when Hammond (5) reported on 25 ovarian biopsies

performed under culdoscopic visualization with only minimal patient morbidity. Steele and associates (17) recommended laparoscopic ovarian biopsy in the investigation of amenorrhea reporting 28 cases they have studied. For some the inadequacy of indirect methods to assess gonadal function was not sufficient to obviate a diagnostic surgical procedure with its associated risks (10, 11, 12, 13, 18). For others a single plasma FSH value was found sufficient to diagnose the presence or absence of ovarian follicles (3). This report is to describe outpatient ovarian biopsies performed by culdoscopy or laparoscopy on 78 amenorrhagic patients in an effort to evaluate their amenorrhea.

METHODS AND MATERIALS

From September 1, 1971 to September 1, 1975 the authors have performed 78 ovarian biopsies on women with primary 35 and secondary 43 amenorrhea chosen from the reproductive endocrinology clinics of the American University Medical Center. The diagnostic workup prior to endoscopy included a complete medical history and physical examination, routine urinalysis, hematological studies and vaginal cytology.

Radiological examinations of the sella turcica as well as visual fields were performed on all cases with secondary amenorrhea and most of the primary amenorrhea cases.

Total urinary gonadotropins were determined by bioassay in 36 patients using the method described by Brown (1) and expressed in Mouse uterine units. LH urinary levels were occasionally estimated by luteinizing hormone immunochemical test supplied by Organon using the 2nd IRP HMG as the standard with values expressed as International units in 24 hour urine with normal ovulatory levels of 150-500 IU/24 hr. Serum gonadotropins FSH and LH were measured on 42 cases by double antibody radioimmunoassay procedures (6, 7) and all samples were performed in the same assay in duplicate. The Second International Reference Preparation of human menopausal Gonadotropin was used as a reference standard for both the FSH and LH assays and expressed in M IU second IRP HMG per milliliter with normal follicular phase levels of 5 to 15 MIU/ml for each. Endocrine tests

Table 1 Gonadotropins and ovarian histology in 35 women with primary amenorrhea

Pat no	Gonadotropins	Gross appearance	Ovarian biopsy
<i>Normal sexual development</i>			
1	Pos 6 12 24 M U	Small Thick capsule	Luteinized stroma Germinal follicles Cystic follicles Thick capsule
2	Pos 6 12 24 48 M U	Small	Normal stroma Germinal follicles Old corpus luteum
3	Pos 6 12 24 M U	Small	Normal stroma Germinal follicles Graafian follicles Corpus albicans
4	Pos 6 12 24 M U	Small	Normal stroma Germinal follicles Graafian follicles
5	Pos 6 12 M U	Small	Normal stroma Germinal follicles
6	Pos 6 12 24 M U	Streak	Normal stroma Few germinal follicles
7	9IU/4hrs	Streak LT Cystic mass RT	LT normal stroma Few germinal follicles RT benign cystic teratoma
8	FSH 1.5 MIU/ml LH 2 MIU/ml	Streak	Normal stroma Germinal follicles
9	Neg 6 M U	Small	Normal stroma Germinal follicles Degenerating graafian follicles
10	Pos 6 M U	Small	Normal stroma Germinal follicles
11	Pos 6 M U	Small	Normal stroma Germinal follicles
12	Neg 6 M U	Small	Normal stroma germinal and graafian follicles undergoing atresia
13	FSH < 2.5 MIU/ml LH 7.5 MIU/ml	Small	Normal stroma Germinal follicles
14	FSH 2 MIU/ml LH 2 MIU/ml	Small	Normal stroma Germinal follicles
15	FSH 10 MIU/ml	Small	Normal stroma Germinal follicles
16	Neg 6 M U	Small Cystic	Normal stroma Germinal follicles Few cystic follicles
17	FSH 2.5 MIU/ml LH 2.1 MIU/ml	Small cystic Thick capsule	Stromal fibrosis Germinal follicles Cystic follicles Atretic germinal follicles
18	Pos 6 M U	Streak	Normal stroma No follicles
19	FSH 2 MIU/ml LH 1.5 MIU/ml	Streak	Fibrous tissue Mesonephric remnants
20	FSH 2.4 MIU/ml LH < 1.5 MIU/ml	Streak	Normal stroma No follicles

(cont)

Gonadotropins	Gross appearance	Ovarian biopsy
FSH 35 MIU/ml LH 27.5 MIU/ml	Streak	Stromal fibrosis No follicles
FSH 46.9 MIU/ml LH 16.5 MIU/ml	Streak	Stromal fibrosis No follicles
<i>Infantilism</i>		
FSH Pos 24 M U LH 33 IU/24hrs	Streak	Fibrous tissue
Pos 48 M U	Streak	Fibrous tissue
<i>Minimal absence of uterus</i>		
FSH 8 MIU/ml LH 15 MIU	Normal	Germinal follicles Graafian follicles
FSH 3 MIU/ml LH 8 MIU/ml	Pos normal	Germinal follicles Graafian follicles
FSH 7.5 MIU/ml LH 3 MIU/ml	Normal	Germinal follicles
FSH 17 MIU/ml LH 18 MIU/ml	Normal	Corpus luteum
FSH 7.5 MIU/ml LH 9 MIU/ml	Normal	Germinal follicles Graafian follicles
FSH 4.2 MIU/ml LH 6 MIU/ml	Normal	Corpus luteum
FSH 1.5 MIU/ml LH 6.15 MIU/ml	Normal	Germinal follicles Graafian follicles
FSH 9.7 MIU/ml LH 10.8 MIU/ml	Normal	Germinal follicles Graafian follicles
FSH 3.6 MIU/ml	Normal	Germinal follicles
FSH 3.75 MIU/ml LH 7.0 MIU/ml	Normal	Germinal follicles Graafian follicles
FSH 8.0 MIU/ml LH 10.6 MIU/ml	Cystic	Cystic follicles Germinal follicles Graafian follicles Luteinized stroma

to thyroid and adrenal functions were done when indicated. Smears stained with toluidine blue were examined for the percentage of Barr bodies according to the technique of Moore and Barr. The karyotypes were determined on peripheral blood lymphocytes by the Moore method (8).

Temperature was documented with basal body temperatures and endometrial biopsies. Laparoscopy was performed on 45 patients under general anesthesia using the following technique. After a skin incision, the culdoscope is inserted and pelvic organs visualized. Then the cul-de sac perforation is made with uterine dressing forceps and a Gutierrez puncture is introduced. Under culdoscopic visualization the puncture is caught with the clamp at its inferior edge. The culdoscope is then removed and the ovary is pulled gently out of the cul-de sac. A suture is taken through the superior border of the ovary for stabilization. By means of a sharp knife a biopsy

is removed under direct vision and the site of biopsy is closed with interrupted chromic sutures. When hemostasis is secured the ovary is replaced in its position. The perforation in the cul-de sac is closed with a figure of eight suture after deflating air from the peritoneal cavity. The patients are then taken to a recovery room and discharged home on the same day on mild analgesics.

Laparoscopy was performed on 33 patients under general anesthesia using the following technique. After securing a safe pneumoperitoneum with two liters of CO₂, a trocar is inserted infraumbilically, an operative laparoscope introduced and pelvic organs visualized. A second hole is made in the right lower abdomen and an ovarian biopsy forceps is inserted. Using the cautery forceps to stabilize the ovary a punch biopsy is taken. The biopsy site is cauterized to secure hemostasis in case of bleeding. The infraumbilical incision is closed with a 4 zero chromic catgut subcuticular suture and the second hole with a simple skin chromic suture.

Table II Ovarian biopsies in 78 amenorrheic women

Primary amenorrhea			35
With follicles			28
Normal follicles		24	
Normal stroma	23		
Luteinized stroma	1		
Fibrosed stroma	-		
Cystic follicles		4	
Normal stroma	1		
Luteinized stroma	2		
Fibrosed stroma	1		
With no follicles			7
Normal stroma	2		
Luteinized stroma	-		
Fibrosed stroma	5		
Secondary amenorrhea			43
With follicles			21
Normal follicles		16	
Normal stroma	15		
Luteinized stroma	1		
Fibrosed stroma	-		
Cystic follicles		5	
Normal stroma		3	
Luteinized stroma	2		
Fibrosed stroma	-		
With no follicles			22
Normal stroma	10		
Luteinized stroma	-		
Fibrosed stroma	12		

The biopsies obtained were sent to pathology where serial sections were made and stained with hematoxylin and eosin. Their sizes were thought to be adequate enough to represent the ovary as a whole measuring 1×0.5 cm on the average. They were assessed with particular reference

Table III Distribution of 78 amenorrheic women and secondary amenorrhea according to the endocrine apparatus and gonadotropin profile

Primary amenorrhea			35
With follicles			28
Normal follicles		4	
Hypergonadotropic	4		
Hypogonadotropic	9		
Normotrophic	10		
Cystic follicles		4	
Hypergonadotropic	1		
Hypogonadotropic	2		
Normotrophic	1		
With no follicles			7
Hypergonadotropic	4		
Hypogonadotropic	3		
Secondary amenorrhea			41
With follicles			16
Normal follicles		16	
Hypergonadotropic	7		
Hypogonadotropic	9		
Cystic follicles		5	
Hypergonadotropic	1		
Hypogonadotropic	4		
With no follicles			25
Hypergonadotropic	21		
Hypogonadotropic	4		

to the germ cell and stromal compartments. The presence of germinal follicles, graafian follicles and atretic follicles, corpora lutea and albicantia was carefully studied.

RESULTS

Primary amenorrhea Table I presents the gonadotropin values and ovarian histology in 35

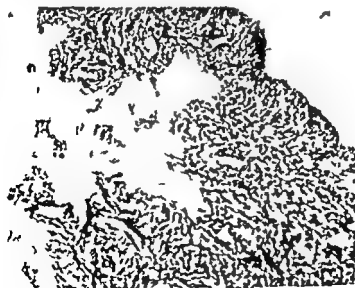
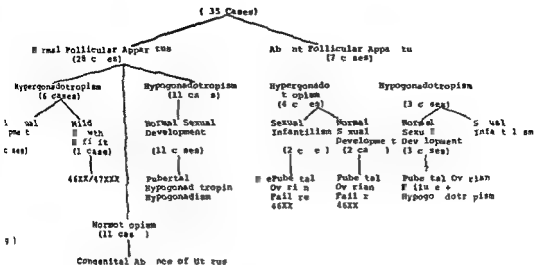


Fig. 1. Squam. germ. cell. follicle in a 14-year-old woman with primary amenorrhea. Normal sexual development and elevated gonadotropins (urinary gonadotropin 44 mouse units) (Original magnification H & E $\times 10$).



Classification of 35 cases of primary amenorrhea on ovarian biopsy

ones of women with primary amenorrhea 22 normal sexual development without prior gen administration 2 with sexual infantilism out hermaphroditism and 11 with congenital nce of the uterus

ble II presents the distribution of 35 wom ith primary amenorrhea according to the logic description of ovarian follicles and na from endoscopic ovarian biopsies. There 8 biopsies with follicles which were normal ordial in 24 and cystic in 4. Those with normal les had a normal stroma except in 1 case where

it was luteinized. Those with cystic follicles had the stroma luteinized in 2 cases and fibrosed in 1.

Table III shows the distribution of these cases according to their gonadotropin profile. 6 were hypergonadotropic in spite of the presence of follicles (Fig. 1). 11 were normotrophic with cystic follicles luteinized stroma in 1. All of them had normal sexual development without prior estrogen stimulation (Fig. 2). 11 cases with normotrophism had congenital absence of the uterus and part or all of the vagina. 1 of them had excessive hirsutism and all had normal sex chromosome patterns. 6 cases with



Fig. 3 Normal ovarian stroma without follicles in an 18 year-old girl with primary amenorrhea, low serum gonadotropins (FSH 2 MIU/ml, LH < 1.5 MIU/ml) and well developed secondary sexual characteristics without prior estrogen stimulation. (Original magnification H & E $\times 25$.)

Table IV Gonadotropins and ovarian histology in 43 women with secondary amenorrhea

Pat no	Gonadotropins	Gross appearance	Ovarian biopsy
<i>With hypergonadotropism</i>			
1	Pos 96 M U LH 1500 IU/24hrs	Small	Normal stroma Germinal follicles
2	Pos 96 M U	Streak	Normal stroma Germinal follicles Corpus albicans
3	Pos 96 M U	Small	Luteinized stroma Germinal follicles
4	Pos 48 M U	Streak	Normal stroma Few atretic follicles
5	Pos 48 M U	Small	Normal stroma Few atretic follicles and Corpora albicantia
6	Neg 6 M U	Small	Normal stroma No follicles
7	Pos 96 M U LH 68 IU/24hrs	Small	Normal stroma No follicles
8	Pos 96 M U	Small	Stromal fibrosis No follicles
9	Pos 48 M U	Small	Normal stroma Few atretic follicles
10	Pos 48 M U LH 35 IU/24hrs	Small	Normal stroma Scanty germinal follicles Early corpus luteum
11	Pos 12 M U	Small	Normal stroma No follicles
12	Pos 12 M U LH >90 IU/24hrs	Small	Normal stroma Germinal follicles
13	FSH >40 MIU/ml LH 36 MIU/ml	Cystic Enlarged Thick Capsule	Stromal thecomatosis Germinal follicles Few cystic follicles
14	FSH 46 MIU/ml LH 25 MIU/ml	Small	Stromal fibrosis No follicles
15	FSH >50 MIU LH 25 MIU/ml	Streak	Stromal fibrosis No follicles
16	FSH >40 MIU/ml LH >40 MIU/ml	Streak	Normal stroma No follicles
17	FSH >40 MIU/ml LH 34 MIU/ml	Small	Stromal fibrosis No follicles
18	FSH >40 MIU/ml LH 15 MIU/ml	Streak	Inactive stroma No follicles
19	FSH >50 MIU/ml LH 22 MIU/ml	Streak	Normal stroma No follicles
20	FSH >50 MIU/ml LH 33 MIU/ml	Small	Normal stroma No follicles
21	FSH >100 MIU/ml LH 24 MIU/ml	Streak	Stromal fibrosis No follicles
22	FSH >40 MIU/ml LH 16 MIU/ml	Small Small	Stromal fibrosis
23	FSH >40 MIU/ml LH 35 MIU/ml	Small	Normal stroma Germinal follicles

IV (cont)

Gonadotropins	Gross appearance	Ovarian biopsy
FSH > 50 MIU/ml LH 22 MIU/ml	Small	Stromal fibrosis No follicles
FSH > 100 MIU/ml LH 31 MIU/ml	Streak	Stromal fibrosis No follicles
FSH > 100 MIU/ml LH 65 MIU/ml	Streak	Normal stroma Focal stromal hyperplasia No follicles
FSH > 40 MIU/ml LH 50 MIU/ml	Small	Normal stroma Corpora albicantia
FSH > 40 MIU/ml LH 71 MIU/ml	Streak	Stromal fibrosis No follicles
FSH > 50 MIU/ml LH > 50 MIU/ml	Streak	Stromal fibrosis No follicles
<i>hypogonadotropism</i>		
Neg 6 M U	Cystic	Thick capsule Normal stroma Cystic follicles
Neg 6 M U	Cystic	Dermoid cyst Thick capsule Normal stroma
FSH Neg 6 M U LH 675 IU/24 hrs	Cystic	Cystic follicles Thick capsule Luteinized stroma Cystic follicles
FSH 13 MIU/ml LH 115 MIU/ml	Cystic	Thick capsule Luteinized stroma Cystic follicles
FSH Pos 6 M U LH 74 IU/24 hrs	Normal	Normal stroma Germinal follicles
FSH 25 MIU/ml LH 475 MIU/ml	Normal	Normal stroma Germinal follicles
FSH 21 MIU/ml LH 72 MIU/ml	Normal	Normal stroma Germinal follicles
FSH 47 MIU/ml LH < 15 MIU/ml	Normal	Normal stroma Germinal follicles Atretic follicles
FSH 42 MIU/ml LH 9 MIU/ml	Normal	Normal stroma Germinal follicles
Neg 6 M U	Normal	Normal stroma Germinal follicles Atrophic follicles
Neg 6 M U	Normal	Normal stroma Germinal follicles Graafian follicles
FSH 15 MIU/ml LH 9 MIU/ml	Normal	Normal stroma Germinal follicles Graafian follicles
Neg 6 M U	Small	Normal stroma No follicles

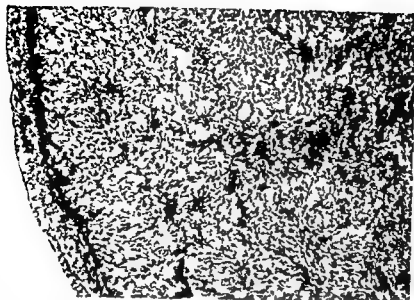


Fig 4 Scanty germinal and stromal thecoma ova in a 13-year-old woman para 3 with amenorrhea of 1 year. Serum gonadotropin: high serum gonadotropin (110 IU/ml LH 36 mIU/ml) magnification H & E $\times 10$

hypergonadotropism had normal sexual development with 46XX sex chromosome patterns except in 1 case who had a mild growth deficit (155 cm 55 kg) and a mosaic sex chromosome pattern with a karyotype on peripheral lymphocytes showing 2% with 47XXX and the rest 46XX. 11 cases with hypogonadotropism had normal sexual development and 46XX on the karyotypes.

There were 7 biopsies of streak gonads without ovarian follicles. 4 were hypergonadotropic with stromal fibrosis and 46XX sex chromatin patterns. Of these 2 had sexual infantilism without hermaphroditism and 2 had their secondary sex characteristics developed without prior estrogen administration.

3 were hypogonadotropic with 46XX sex chromosome complement and developed secondary sex characteristics (Fig 2). Ovarian stroma normal in 2 (Fig 3) fibrotic in 1.

Secondary amenorrhea. Table IV presents gonadotropin values and ovarian histology in 2 categories of women with secondary amenorrhea: 29 with elevated gonadotropins and 14 with low gonadotropin levels.

Table II presents the distribution of 43 women with secondary amenorrhea according to the histologic description of ovarian follicles. The stroma from endoscopic ovarian biopsies. There were 21 biopsies with follicles which were normal



Fig 5 Scanty germinal cells and an early corpus luteum in a 13-year-old woman with secondary amenorrhea of 3 years. Serum gonadotropin: high gonadotropin (110 IU/ml LH 36 mIU/ml) magnification H & E $\times 10$

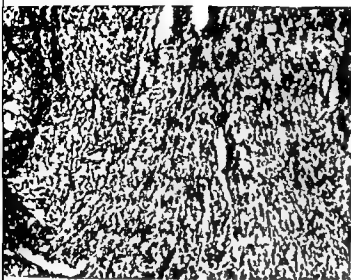


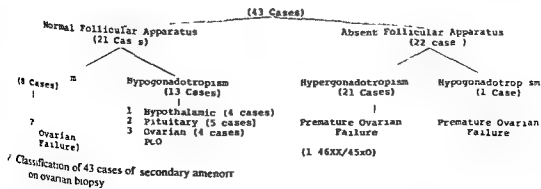
Fig 1 Hyperplasia of theca interna and stromal luteinization in a 24-year-old woman with secondary amenorrhea of 4 years duration who failed to ovulate on clomid (Original magnification H & E $\times 10$)

and cystic in 5. Those with normal follicles had normal stroma except in 1 case where it was fibrosed. Those with cystic follicles had a normal stroma in 3 cases and a luteinized one in 2. Table III shows their distribution according to gonadotropin profile. 8 cases were hypergonadotropic in spite of the presence of follicles, stromal atrophy in 1 (Fig 4) and an early corpus luteum in another (Fig 5). 13 cases were gonadotropotropic with cystic follicles in 4 who also had stromal luteinization (Fig 6). There were 22 biopsies without ovarian follicles, a normal stroma in 10 and a fibrosed stroma in 12 of them (Table II). All except 1 were hypergonadotropic (Table III). In the light of endoscopic and histologic findings it is possible to distribute patients with primary

amenorrhea into 2 main groups in relation to subsequent management (Fig 2). There were 17 cases with ovarian follicles and no müllerian duct abnormalities. Human menopausal gonadotropins were administered to 5 of them who had high endogenous gonadotropin levels and was successful in 1 evidenced by a biphasic temperature chart while it failed in 4 even on high dose schedules.

HMG was also administered to 7 of them who had low gonadotropins and was successful in 2 only. It failed in 2 cases who had cystic follicles even with very high dose schedules. In 1 of the patients a Y chromosome was present. She refused surgery for removal of the gonads. Estrogens were administered to those with sexual infantilism and secondary sexual development.

Patients with secondary amenorrhea were also



distributed into 2 groups (Fig. 7). 21 cases had ovarian follicles. Human menopausal gonadotropins were administered to 4 of them who had high endogenous gonadotropins but failed to induce ovulation in any, including 1 case who had an early corpus luteum on a biopsy done just prior to the initiation of therapy.

13 cases with hypogonadotropism were all refractory to clomid and gonadotropin therapy. 9 had a central pathology. 1 of them got pregnant on ergocryptin and 1 had a pituitary adenoma that was revealed by radiology a year following the biopsy. It was removed transphenoidally. 4 had polycystic ovaries, 1 of whom got pregnant after wedge resection.

The 78 ovarian biopsies performed were all uneventful without complications except in 1 case which following the biopsy by culdoscopy had bleeding from the ovarian site that could not be controlled through the vaginal incision. A laparotomy was performed and on the 5th hospital day she was discharged in good condition.

DISCUSSION

The fertility prognosis of many amenorrheic women has changed considerably since agents for the induction of ovulation have become available. However, the therapy is expensive and entails some undesirable side effects. Hence accurate assessment of ovarian histology can be helpful in the selection of patients and in anticipating their therapeutic responses. Patients with primary amenorrhea who have normal sexual development with out prior estrogen stimulation and with no müllerian duct abnormalities could either have high or low endogenous gonadotropins. Out of 14 such cases with hypogonadotropism, ovarian biopsies revealed the absence of follicles in 3. Gonadotropins could have been given to these patients to no avail. The only way we could explain their amenorrhea was by assuming a central as well as an ovarian pathology manifested through hypogonadotropism plus pubertal ovarian failure. 8 other cases with hypergonadotropism revealed the absence of follicles in 2 and their presence in 6. Those who had no follicles should have been producing enough estrogens at one time to account for their normal secondary sexual characteristics. The follicles they could have had should have undergone atresia and disappeared before the biopsies were performed.

Their amenorrhea would be due to a premature ovarian failure in contrast to the premenopausal state which is characterized by sexual infatuation. The 6 cases with follicles had a similar ovarian histology according to the cases reported by Jones (4) and Jones (5). They all had numerous primordial follicles, 10 antral graafian follicles in 2 and an old corpus luteum in 1. There was evidence also of increased LH stimulation in 1 case who had stromal luteinization and cystic follicles. Their ovaries however did not seem to be completely resistant to gonadotropin stimulation, endogenous or exogenous, evidenced by an old corpus luteum in 1 and the poor response of another case to HMG therapy. The reason for a decreased sensitivity of these ovaries and an altered feedback effect that causes decreased gonadotropin production remains to be established.

Patients whose ovaries fail prematurely cannot be distinguished clinically from those with other causes of secondary amenorrhea. Although the diagnosis is almost certain if the excretion of gonadotropins is increased. Yet ovarian biopsies performed on such cases revealed the presence of ovarian follicles in 8 of them with an early corpus luteum in 1 and cystic follicles in another case. 7 of them showed evidence of increased LH stimulation and stromal luteinization (9, 16). The infertility of these patients and their refractoriness to exogenous gonadotropins speak for an altered feedback function. An ovarian biopsy however would allow the physician to give an accurate prognosis for a serious diagnosis. Whether an ovarian biopsy ought to be considered in patients with secondary amenorrhea who have low endogenous gonadotropins and who are refractory to LH stimulation depends on the availability of laboratory methods to delineate those with hypergonadotropism from those with pituitary or ovarian pathology. We believe however that certain cases of polycystic ovary syndrome who show hyperandrogenism on histology may benefit from an ovarian wedge resection.

REFERENCES

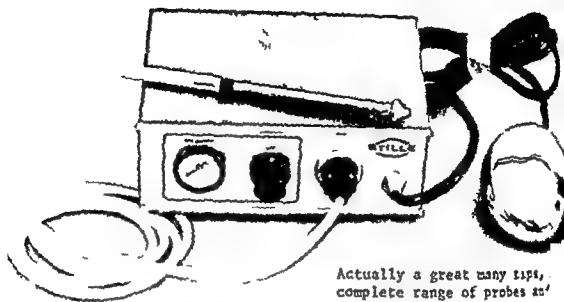
1. Brown P. S. The assay of gonadotropins in the urine of nonpregnant human subjects. *J Endocrinol* 11: 1956.
2. Clyman M. J. Operative culdoscopy. *Obstet Gynecol* 37: 840 1969.
3. Goldenberg R. L., Grodin J. M., Rabinowitz M. Gonadotropins in women with amenorrhea. *Am J Obstet Gynecol* 116: 1001 1971.

- ones G S & Moraes Ruehsen M A new syndrome of amenorrhea in association with hypergonadotropism and apparently normal ovarian follicular apparatus *Am J Obstet Gynecol* 104 597 1969
- Marshall J R & Hammond C B Ovarian biopsy performed under culdoscopic visualization *Am J Obstet Gynecol* 96 1077 1966
- Midgley A M Radioimmunoassay A method for human chorionic gonadotropin and human luteinizing hormone *Endocrinology* 79 10 1966
- Moorhead P S Nowell R C & Melfman W J et al Chromosome preparations of leukocytes cultured from human peripheral blood *Exper cell Res* 20 613 1960
- Moraes Ruehsen M & Jones G S Premature ovarian failure *Fertil Steril* 18 440 1967
- Penny R Guyda H J Baghdassarian A et al Correlation of serum follicular stimulating hormone (FSH) and luteinizing hormone (LH) as measured by radioimmunoassay in disorders of sexual development *J Clin Invest* 49 1847 1970
- Reschini E Guistina G Dalberton A et al Radioimmunoassayable plasma luteinizing hormone in primary amenorrhea *Am J Obstet Gynecol* 111 173 1971
- Ryan R J Cloutier M D Hayles A B et al The clinical utility of radioimmunoassay for serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) *Med Clin North Am* 54 1049 1970
- Schlach D M Parlow A F Boon E C et al Measurement of human luteinizing hormone in plasma by radioimmunoassay *J Clin Invest* 47 665 1968
- Shearman R J A physiologic approach to the differential diagnosis and treatment of primary amenorrhea *J Obstet Gynaecol Br Commonw* 75 1101 1968
- Starup J Sele V & Hennksen B Amenorrhea associated with increased production of gonadotrophins and a morphologically normal ovarian follicular apparatus *Acta Endocrinologica* 66 748 1971
- Starup J & Sele V Premature ovarian failure *Acta Obstet Gynecol Scand* 52 259 1973
- Steele S J Bealby D M & Papadakis L Visualization and biopsy of the ovary in the investigation of amenorrhea *Obstet Gynecol* 36 899 1970
- Taymor M L Management of amenorrhea (edited by C J Collins) p 70 Charles C Thomas Springfield Ill

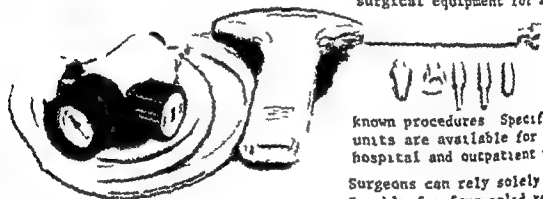
Submitted for publication Feb 6 1978

Karam Karam
Department of Obstetrics and Gynecology
American University Hospital
Beirut
Lebanon

Spembly has a little tip for all cryosurgeons



Actually a great many tips, complete range of probes and technical leadership in cryosurgical equipment for all



known procedures. Specific units are available for off-hospital and outpatient use.

Surgeons can rely solely on Spembly for four solid reasons:

- excellence of equipment performance and design
- world wide experience and distributor network
- comprehensive technical and instructive literature
- and the knowledge that Spembly is continually developing cryosurgical applications and technical

If you would like more information and literature on the Spembly range, please contact your local Spembly supplier or write to us in Andover. Spembly Limited, Newbury Road, Andover, Hampshire SP10 4DR, England.

Telephone (0264) 65741 Telex 61601

Or to **STILLE**

SWEDEN AB STILLE-WERNER, Box 43051, S-100 72 Stockholm
FINLAND OY STILLE AB, Nervanderinkatu 5 D,
SF-00100 Helsinki 10

NORWAY STILLE A S, Postboks 61 Leirdal, Oslo 10
SWITZERLAND STILLE AG Postfach, CH-8038 Zürich

Spembly
FIRST CHOICE
IN CRYOSURGERY

CAP, HCS AND URINARY OESTRIOL ASSAYS IN DIABETIC PREGNANCY

Gunnar Rydén and Goran Berg

From the Department of Obstetrics and Gynecology, University Hospital, Linköping, Sweden

Abstract. The value of serial estimations of plasma CAP and urinary oestriol assays in pregnancies complicated with diabetes has been studied. The material consisted of 31 patients, 16 of whom delivered normal infants and 15 delivered newborns with diabetic foetopathy. It is concluded that the levels of CAP, HCS and urinary oestriol excretion in diabetic pregnancies are comparable to those found in normal pregnancy. No significant differences in the biochemical parameters were detected between diabetic women who delivered infants with foetopathy compared to diabetic women who delivered normal infants. The maternal oestriol excretion was, however, somewhat higher in diabetic women who delivered infants with macrosomia.

Kleiner et al. (7) concluded that CAP assays should be used in association with oestriol assays when assessing risk pregnancies, such as diabetes. Chapman et al. (11) found higher CAP values in diabetic pregnancies compared to normal pregnancies, whereas no such difference was observed for urinary oestriol assays.

The purpose of the present investigation was to study the plasma CAP, HCS and urinary oestriol levels in diabetic pregnancy, with special reference to the ability to predict diabetic foetopathy.

MATERIAL

The material consisted of 31 patients with insulin-requiring diabetes. They were followed by serial assays of CAP, HCS and urinary oestriol from the 32nd week of pregnancy until delivery. Plasma CAP and HCS assays were performed at least once a week. Urinary oestriol assays were performed daily or every other day but only assays performed on the same day as the CAP and HCS assays are included in this study.

The patients were divided into two groups: one of 16 patients who delivered normal infants (within ± 2.5 SD for the gestational age) and a second of 15 patients who delivered infants with signs of foetopathy. The diagnosis was confirmed by a paediatrician according to the usual clinical criteria. Patients with toxicemia, intrauterine growth retardation and hyaline extension were excluded from the present study, as these conditions per se can influence the biochemical values.

The diabetic women were managed according to the principles described by Moll (10). The patients were admitted to the hospital at the beginning of the pregnancy for regulation of the blood glucose, with an outpatient follow-up every second week. A second hospital stay was arranged in the 34th week of pregnancy. From the 36th week of pregnancy until delivery the patients had daily glucose estimations at least five times a day (1, 2, 3, 4, 5, 10 and 11) and urinary glucose was measured four times a day.

Recent study (14) dealt with the value of serial estimations of cystine aminopeptidase (CAP), human chorionic somatomammotrophin (HCS), maternal plasma and maternal urinary oestriol in risk pregnancies, such as preeclampsia, toxemia and intrauterine growth retardation. It is concluded that all three tests can yield information about the condition of the fetus and/or placenta and that more accurate information concerning the fetoplacental unit can be gained by combining oestriol assays with CAP or HCS assays. In diabetic pregnancies are accompanied by a high incidence of complications in the newborn. Numerous studies have demonstrated the clinical value of urinary oestriol determinations in diabetic pregnancies (see Persson et al. (12)). The value of oestriol estimations is less clear. Some authors (16, 18) reported high HCS values in diabetic pregnancies, whereas others (3, 9, 13) have found normal values. The diabetogenic properties of HCS and the levels of this hormone of special interest in pregnancies complicated by diabetes. Reports on estimations in diabetic pregnancies are few.

Table I Weekly maternal blood glucose levels (mean \pm S D)

Pregnancy week	Normal infant group		Diabetic foetopathy group	
	n	mmol/l	n	mmol/l
32	9	5.9 \pm 1.17	8	6.7 \pm 1.68
33	10	5.6 \pm 1.00	11	6.2 \pm 1.27
34	14	5.6 \pm 1.08	17	6.1 \pm 1.64
35	14	5.6 \pm 1.40	13	6.2 \pm 2.02
36	15	5.4 \pm 0.96	13	5.4 \pm 1.13
37	15	5.2 \pm 1.13	15	6.0 \pm 1.69
38	14	4.7 \pm 0.75	14	5.2 \pm 0.75
39	11	4.7 \pm 0.95	7	5.1 \pm 0.95

Table II Mean value of the 1st and 5th standard deviation of the blood glucose (mean \pm S D)

Pregnancy week	Normal infant group		Diabetic foetopathy group	
	n	mmol/l	n	mmol/l
32	9	2.5 \pm 0.44	8	6.14
33	10	2.6 \pm 0.75	11	7.43
34	14	2.5 \pm 0.63	17	5.31
35	14	2.3 \pm 0.93	13	4.19
36	15	2.3 \pm 0.64	13	7.10
37	15	2.3 \pm 0.80	15	2.81
38	14	1.9 \pm 0.69	14	11.08
39	11	2.1 \pm 0.83	7	11.08

METHODS

Urinary oestriol assays were made according to Hainsworth & Hall (4). Plasma cystine aminopeptidase was determined according to Peeters (11), later modified according to Tovey (20). A comparative study of the two methods performed on 106 samples gave a correlation coefficient of 0.96. The calculation of CAP activity was performed according to Tovey (10). Plasma human chorionic somato-mammotrophin was measured according to Leitchworth et al. (18).

RESULTS

An analysis of the maternal blood glucose levels from the 32nd week of pregnancy until delivery is reported in Table I. The mean maternal blood glucose level for each week, calculated from the five daily blood glucose estimations performed, demonstrates the generally higher levels among patients who delivered infants with diabetic foetopathy. The weekly individual standard deviation has also been compared to see if diabetic women with foetopathic newborns have greater fluctuations in blood glucose levels. As shown in Table II no

significant difference was found between the groups.

The mean birth weight of the infants, the gestational age, Apgar score after 1 and 5 min, and the lowest blood sugar in the neonatal period are presented in Table III. There was no perinatal mortality. The increased incidence of hypoglycaemia observed in diabetic foetopathy (5) could not be demonstrated in the present study. This is probably explained by the management of the newborn with early feeding.

The results of the biochemical tests are presented in Fig. 1. For comparison the figure is divided into 3th and 95th percentile levels in normal pregnancy quoted from Rydén & Adgeblad (14). The results obtained in diabetic pregnancy are comparable to normal pregnancy. Concerning CAP and HCS no difference was observed between the two groups. Infants with diabetic foetopathy had somewhat higher oestriol values than the normal group.

A regression analysis was performed to study the relationship between the biochemical tests and

Table III Birth weight, gestational age, Apgar score after 1 and 5 min and lowest blood sugar in newborns

Mean value \pm S D

	n	Gestational age (weeks)	Birth weight (g)	Apgar score		Lowest blood sugar (mmol/l)
				1 min	5 min	
Normal infants	16	39.3 \pm 1.39	3.731 \pm 474	8.6 \pm 0.70	9.1 \pm 0.4	1.8-0.63
Diabetic foetopathy	15	38.9 \pm 1.19	3.949 \pm 478	8.0 \pm 0.23	9.4 \pm 0.61	1.9-0.44

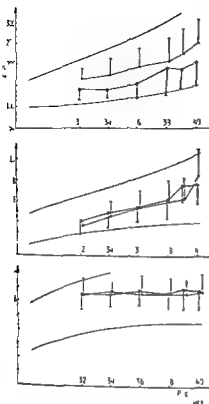


Fig. 1. Mean plasma CAP, HCS and urinary oestriol values (\pm SD) in diabetic women who delivered normal infants (\times) and infants with diabetic foetopathy (\bullet). The 5th and 95th percentile levels in normal pregnancy are also given.

birth weight and placental weight respectively. The last value before delivery was compared with the weight of the placenta and the infant birth weight. In patients who delivered normal infants a relation was found between urinary oestriol excretion and infant birth weight ($r=0.49$, $P=0.02$). However urinary oestriol excretion correlated neither with placental weight ($r=0.69$, $P=0.002$) nor with CAP correlated to placental weight ($r=0.52$, $P=0.01$) but not to infant birth weight, whereas no relation was found between HCS and either placental or birth weight. In pregnancies complicated by diabetic foetopathy no correlation was found between either placental or infant weight and the biochemical tests studied.

DISCUSSION AND CONCLUSION

Urinary oestriol assays are generally accepted as a valuable method for assessing the condition of

the foetoplacental unit in diabetic pregnancy (13). The value of placental function tests such as CAP and HCS is less clear. Chapman et al. (1) observed higher CAP values from 30 weeks onwards in diabetic pregnancy compared to normal pregnancy and suggest that CAP assays might be a valuable parameter in evaluating the condition of the foetus in poorly controlled diabetic pregnancies. This could not be confirmed in the present study. The CAP values in the patients who delivered babies with diabetic foetopathy did not differ from those in patients who delivered normal infants. Both high (15–16–18) and normal HCS values (9–13) have previously been reported in diabetic pregnancies. Other authors (2, 19–21) have found high HCS values in pregnant patients with poorly controlled diabetes and normal values in well-controlled diabetes. The diabetogenic property of HCS is well established (6). High and increasing HCS values could possibly influence the maternal glucose metabolism and give rise to a more intractable blood glucose level and hence a greater risk of diabetic foetopathy. Tyson & Hock (20) have proposed that the HCS levels per se can be responsible for the imbalance of blood glucose homeostasis that is often seen in diabetic pregnancy. The present results as well as the finding of Spellacy & Cohn (16) that the rising insulin requirements in diabetic pregnancy are not related to the increasing production of HCS do not support the theory proposed by Tyson & Hock.

The following conclusions can be drawn. Neither CAP nor HCS are of value in predicting the condition of the foetus at birth with respect to diabetic foetopathy. The levels of CAP and HCS in diabetic pregnancy are comparable to those found in normal pregnancy.

Prenatal loss in late diabetic pregnancy is seldom dependent on the placenta, the usual cause being a derangement of the glucose homeostasis which exerts its effect directly on the foetus. The production of oestriol during pregnancy is primarily dependent on foetal adrenal function, so that changes in the condition of the foetus are reflected in the maternal oestriol excretion, hence the clinical value of serial oestriol assays in diabetic pregnancy. However, if the diabetic pregnancy is complicated by toxæmia or intrauterine growth retardation, both HCS assays (13) and CAP assays (7–14) can be useful tools besides oestriol assays for monitoring the foetus.

REFERENCES

- 1 Chapman L, Silk E, Skupny A & Tooth E A Spectrofluorimetric assays of serum cystine aminopeptidase in normal and diabetic pregnancy compared with total oestrogen excretion *J Obst Gynecol Br Commonw* 78 435 1971
- 2 Cohen M, Haour F, Dumont M & Bertrand J Prognostic value of human chorionic somatomammotrophin plasma levels in diabetic patients *Am J Obstet Gynecol* 115 207 1973
- 3 Genazzani A R, Cocola F, Casoli M, Mello G, Scarselli G, Neri P & Fioretti F Human chorionic somatomammotrophin radioimmunoassay in evaluation of placental function *J Obstet Gynecol Br Commonw* 78 577 1971
- 4 Hainsworth I R & Hall P E A simple automated method for the measurement of oestrogens in the urine of pregnant women *Clin Chim Acta* 35 201 1971
- 5 Isles T E, Dickson M & Farquhar J W Glucose tolerance and plasma insulin in newborn infants of normal and diabetic mothers *Pediatr Res* 9 198 1968
- 6 Kaplan S L Human chorionic somatomammotrophin secretion biologic effects and physiologic significance *In The Endocrine Milieu of Pregnancy Puerperium and Childhood* (ed R B Jaffe) p 75 Ross Laboratories Columbus Ohio 1974
- 7 Kleiner H, Stavric V I, Brouet Yagh M, Schwens J & Graff G L A Mesure de l'oxytocinase plasmatique *J Gynecol Obstet Biol Reprod* 5 25 1976
- 8 Letchworth A T, Boardman R J, Bristow C, Landon J & Chard T A rapid semiautomated method for the measurement of human chorionic somatomammotrophin The normal range in the third trimester and its relation to fetal weight *J Obstet Gynaecol Br Commonw* 78 542 1971
- 9 Lindberg B S & Nilsson B A Human placental lactogen (HPL) levels in abnormal pregnancies *J Obstet Gynaecol Br Commonw* 80 1046 1973
- 10 Møller E B Studies in Diabetic Pregnancy *Stu dentlitteratur* Lund 1970
- 11 Peeters J A B M Automated determination of serum oxytocinase activity *Clin Chem* 18 563 1973
- 12 Persson B, Lunell N-O, Carlström K & Fuschjelm M Urinary oestrol excretion in strictly controlled diabetic pregnancies *Acta Obstet Gynecol Scand* 49 379 1970
- 13 Persson B, Lunell N O, Aubert M L, Carlström K & Felber J P Determination of plasma human chorionic somatomammotrophin and urinary oestrol in diabetic pregnancies *Acta Obstet Gynecol Scand* 52 111 1973
- 14 Rydén G & Kågedal H CAP HCS and urinary oestrol measurements in risk pregnancies—a comparative study *J Perinatal Medicine* 5 744 1977
- 15 Saaman N A, Bradbury J & Goplerud C Serol hormonal studies in normal and abnormal pregnancy *Am J Obstet Gynecol* 104 781 1969
- 16 Saxena B N, Emerson K & Selenkow H A Serum placental lactogen (HPL) levels as an index of placental function *N Engl J Med* 281 125 1969
- 17 Spellacy W N & Cohn J E Human placental lactogen levels and daily insulin requirements in patients with diabetes mellitus complicating pregnancy *Obstet Gynecol* 42 330 1973
- 18 Spellacy W N, Buhl W C, Berk S A & McCreary S A Distribution of human placental lactogen in the last half of normal and complicated pregnancies *Am J Obstet Gynecol* 120 714 1976
- 19 Spona J & Janisch H Serum placental lactogen (HPL) as index of placental function *Acta Endocrinol* 68 401 1971
- 20 Tovey J E, Dawson P J G & Fellowes K P Evaluation of S-benzyl l-cysteine-4-nitroamide as substrate for serum cystine aminopeptidase *Clin Chem* 19 756 1973
- 21 Tyson J E & Hock R A Gestational and pregestational diabetes An approach to therapy *Am J Obstet Gynecol* 125 1009 1976
- 22 Ursell W, Brudenell M & Chard T Placental lactogen levels in diabetic pregnancy *Br Med J* 1973

Submitted for publication May 5 1977

Gunnar Rydén
Dept of Obstetrics and Gynecology
University Hospital
S 581 85 Linköping Sweden

THE EFFECTS OF ISOPROTERENOL ON FETAL OXYGENATION

R E Myers I Joelsson and K Adamsons

*From the Laboratory of Perinatal Physiology
National Institute of Neurological and Communicative
Disorders and Stroke National Institutes of Health Bethesda Maryland USA
the Department of Obstetrics and Gynecology Umeå University Umeå Sweden
and the Department of Obstetrics and Gynecology Brown University
Providence Rhode Island USA*

act Infusion of isoproterenol ($1 \mu\text{g/kg/min}$ i.v.) into anesthetized pregnant rhesus monkey near term consistently reduced fetal oxygenation despite diminishing uterine activity. The decline in pO₂ of fetal arterial blood (mean $= 4.3 \pm 3 \text{ mmHg S.D.}$) was accompanied by a decrease in pCO₂ tension (mean $= 4.6 \pm 7 \text{ mmHg}$) and a decrease in pH (mean $= 7.04 \pm 0.02 \text{ S.D.}$). There was an increase in heart rate and a widening of pulse pressure in the fetus and also in the adequately oxygenated fetus providing evidence that the agent crosses the placenta. The hypoxia oxygenated fetuses developed bradycardia and hypotension. Administration of isoproterenol directly to the fetus elicited similar changes in the composition of blood and in blood pressure and heart rate to those noted after administration of the agent to the mother.

A variety of synthetic beta receptor stimulating agents have been used during the last five years to delay patients in premature labor (1-4) and to treat fetal asphyxia produced by excessive uterine activity (5). When these agents are administered to laboratory primates they have been found to reduce the frequency of uterine contractions both in spontaneous and in induced labor (6). These same agents also alter the conductance of blood vessels throughout the body and thus modify the distribution of blood flow. Because the blood vessels of the fetus like those of other viscera are poorly supplied with beta adrenergic receptors administration of beta adrenergic agents could reduce uterine blood flow and hence impair oxygenation of the fetus. This would pertain particularly if the agent decreases mean arterial blood pressure of the mother which limits compensatory vasoconstriction in the uterine circulation. Because the infusion of epinephrine into the pregnant primate is known to cause asphyxia of the fetus (7) it seemed of interest to determine the

effects of a pure beta receptor stimulating agent.

Currently available clinical literature does not provide information as to the effects of beta adrenergic receptor stimulating agents on the oxygenation of the fetus. When these agents are used in the treatment of premature labor the fetus is not usually accessible for blood sampling. However there is a study in which a slight reduction in fetal pH was observed when nitroglycerine was infused into the mother for longer than 30 minutes (8). When beta adrenergic stimulating agents are used to suppress excessive uterine activity the effects of this reduction in uterine activity on the oxygenation of the fetus cannot be separated from those brought about by the action of these drugs on the conductance of the blood vessels supplying the uterus and other organs. Beta mimetic drugs administered to the mother could affect the oxygenation of the fetus by altering the blood flow through the umbilical vessels after their transfer to the fetus.

The present study was conducted under conditions of low initial uterine activity in order to focus specifically on those alterations in fetal oxygenation that result from changes in the circulatory state of the mother and fetus.

MATERIAL AND METHODS

Fifteen rhesus monkeys in the last decile of pregnancy were used. Isoproterenol was administered to the mother by constant i.v. infusion on 13 occasions in 11 animals to the fetus on 9 occasions in 7 animals and finally to the mother by rapid i.v. injection 6 times in 4 animals. In every instance a rest period lasting from 55 to 90 min was allowed between infusions or injections to the same animal to permit a full recovery of the function of the cardiovascular system and of the composition of the blood

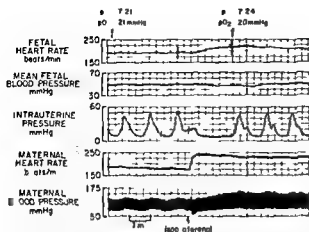


Fig 1 Fetal and maternal heart rates and blood pressures and intrauterine pressure before and after rapid injection of isoproterenol ($8 \mu\text{g/kg i.v.}$) to the anesthetized pregnant rhesus monkey near term. Note the increase in heart rate and the slight fall in blood pressure in the presence of an essentially constant state of oxygenation

back to baseline values. The duration of pregnancy was known for each animal with an accuracy of ± 2 days. The animals' body weights varied from 6.2 to 9.4 kg.

Hysterotomy was performed under pentobarbital anesthesia (35 mg/kg i.v.). Polyethylene catheters were inserted into the femoral artery and vein of the mother and of the fetus to record blood pressure and heart rate, and to withdraw blood samples as well as to administer isoproterenol. The intrauterine pressure was recorded by a catheter placed into the amniotic cavity. All samples of maternal and fetal arterial blood were analyzed for pH, pO_2 and pCO_2 using appropriate microelectrodes (London Company). Throughout the experiment the oxygen tension of maternal arterial blood was maintained in the range of 90 to 120 mmHg by adjustments of the oxygen concentration of the inspired gas mixture. The mother's deep colonic temperature was controlled in the range of 37 to 38°C using a servo modulated overhead heat shield.

During the control period each mother was given 250 ml of 5% dextrose in water by i.v. drip. The blood volume of the fetus was maintained throughout the study period by replacing the blood withdrawn with an equivalent volume of maternal blood. A more detailed account of all procedures used has been given earlier (7).

The isoproterenol solutions were prepared fresh to contain $10 \mu\text{g/ml}$ of the base in isotonic saline. The drug was administered through the femoral vein catheter of the mother or fetus either by rapid injection or by infusion at a constant rate over a 20-min period. In a single instance the infusion was discontinued before 70 min elapsed because of the threat of imminent death of the fetus due to severe asphyxia. The dosage for isoproterenol infusion to the mother was $1 \mu\text{g/kg/min}$ and to the fetus 0.5 to $2.0 \mu\text{g/kg/min}$. The dosage for rapid injection into the mother ranged from 2 to $8 \mu\text{g/kg}$. In calculating the dose for infusion directly into the fetus the combined weights of the fetus and placenta were assumed to be 500 gm.

RESULTS

Control values of the mothers and fetuses prior to administration of isoproterenol

The maternal heart rates under anesthesia ranged from 125 to 175 beats/min while their blood pressures ranged from 120/75 to 170/110 mmHg. The maternal pH, pO_2 and pCO_2 values while they were breathing spontaneously ranged from 7.37 to 7.44, 84 to 108 mmHg and 28 to 38 mmHg respectively. Uterine activity was slight to moderate in all instances. The so-called resting tone ranged from 7 to 8 mmHg, the intervals between contractions ranged from 3 to 12 min and the amplitudes of the contractions ranged between 2 and 30 mmHg. In most instances the amplitudes of contractions remained less than 10 mmHg.

The fetal heart rates ranged between 175 and 225 beats/min while the blood pressures ranged from 51/33 to 70/40. The pH values of blood obtained from the fetal lower thoracic aorta averaged 7.27 with a range of 7.20 to 7.37. The mean pO_2 was 45 with a range of 19 to 33 mmHg and that of pCO_2 was 45 with a range of 38 to 47 mmHg. The mean values of red cell concentrations of maternal and fetal arterial bloods were 35% and 45% with ranges of 31 to 37% and 42 to 46% respectively.

Maternal and fetal responses to rapid injection of isoproterenol to the mother

Isoproterenol was administered directly to the mother by rapid injection in a dose which ranged from 2 to $8 \mu\text{g/kg}$ in 6 instances in 4 animals. These injections in this dose range produced a rapid increase in maternal heart rate which ranged from 10% to 60% (see Figs 1, 2 and 3). In 5 of the 6 instances the systolic pressure rose from 9 to 36%, while the diastolic pressure changed variably from -17 to $+10\%$. In the remaining animal the injection caused an initial decrease of 7% in both the systolic and diastolic blood pressure. In those instances where regular intermittent contractions of the uterus were present the injection of isoproterenol increased the interval between contractions but had no effect on the amplitude. The effects on heart rate, blood pressure and uterine activity were transient and preinjection values were restored within 15 min. Maximal changes in heart rate and blood pressure were attained already at a dose of $2 \mu\text{g/kg}$.

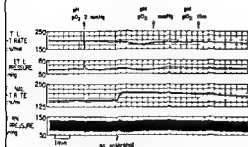


Fig 2 Fetal and maternal heart rates and blood pressures during injection of isoproterenol ($2 \mu\text{g/kg}$) into the preterm rhesus monkey near term. Note transient decrease in fetal heart rate and blood pressure and in pH and pO_2 of fetal arterial blood.

The rapid injection of isoproterenol into the mother caused an increase in fetal heart rate similar to that documented in Fig 1 in 4 of 6 instances. This rise in fetal heart rate lasted from 5 to 10 min and could be detected within 40 seconds after the isoproterenol was administered to the mother. The pCO_2 and pH values of fetal arterial blood sampled in 5, 10 and 20 min after injection showed no significant changes. However, in two instances the injection of isoproterenol to the mother led to clearly defined episodes of bradycardia and hypotension in the fetuses. These values recovered to their preinjection values by the 5 min blood samples were drawn. When fetal arterial blood of these two fetuses was sampled during the period of maximal bradycardia, significant decreases in pO_2 were observed. In one the pO_2 decreased from 71 to 17 in one and from 13 to 9 mmHg in the other. The arterial blood pO_2 s returned to their preinjection values within 10 min in both instances (See Figs 2 and 3).

and fetal response to infusion of isoproterenol into the mother

Isoproterenol was administered to the mother by infusion ($1 \mu\text{g/kg/min}$) on 13 occasions in 11 monkeys. These infusions produced a prompt increase in heart rate of the mother with a mean of 34% and a standard deviation of $\pm 10\%$. Maternal systolic blood pressure increased transiently by a mean value of $14 \pm 4\%$ S.D. Diastolic blood pressure decreased by a mean of $9 \pm 6\%$ S.D. When definite uterine contractions were present the intercontraction interval

was prolonged by up to two-fold. At the same time the amplitudes of the uterine contractions were altered only slightly or not at all. These effects on the myometrium were more marked at the beginning than toward the end of the period of infusion. The pO_2 of maternal arterial blood decreased in all instances. This decrease ranged from 19 to 45 mmHg. To compensate for this decrease the oxygen concentration in the inspired gas mixture of the mother was increased until the maternal arterial blood samples showed a pO_2 that was restored to the preinfusion values in all instances. The pCO_2 and pH values of maternal arterial blood did not change significantly. The maternal cardiovascular changes were maximal during the early phases of infusion and the values generally recovered to preinjection levels within 10 min of termination of infusion.

The infusion of isoproterenol to the mother caused no changes in blood pressure or heart rate of the adequately oxygenated fetus. However, it did lead to reductions in fetal aortic blood pO_2 in all instances. This decrease ranged from 2 to 8 mmHg with a mean value of 4.3 and a standard deviation of ± 2.3 mmHg. This decrease in oxygen pressure of fetal blood was accompanied by a mean increase in pCO_2 of 4.6 ± 2.7 mmHg S.D. and a mean decrease in pH of 0.04 ± 0.02 S.D. The composition of fetal

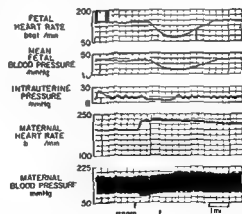


Fig 3 Fetal and maternal heart rates and blood pressures and intrauterine pressure after a rapid injection of isoproterenol ($2 \mu\text{g/kg}$ i.v.) to anesthetized rhesus monkey near term with severely acidotic and hypoxic fetus ($\text{pH}=6.83$, $\text{pO}_2=13$ mmHg). Note the induction of transient bradycardia and hypotension in the fetus despite a decrease in uterine activity. The changes in vital signs were associated with a fall of pO_2 in fetal arterial blood from 13 to 9 mmHg.

arterial blood recovered to its preinfusion values in all instances within 20 min after drug infusion was terminated

Fetal response to infusion of isoproterenol to the fetus

Isoproterenol was infused intravenously directly to the fetus in 9 instances in 7 animals in the dose range of 0.5 to 2.0 $\mu\text{g/kg/min}$. These infusions led to average increase in fetal heart rate of $15\% \pm 7\%$ S.D. This was accompanied by small and variable changes in fetal systolic blood pressure (mean = $2.6 \pm 5.5\%$ S.D.) and consistent decreases in diastolic blood pressure which ranged from 13 to 32% (mean = $16.7 \pm 6.1\%$ S.D.). These alterations in fetal cardiovascular function were accompanied by decreases in fetal pO_2 (mean = 5.9 ± 2.9 mmHg S.D.) and pH (mean = 0.07 ± 0.03 S.D.) and increases in pCO_2 (mean = 7.6 ± 5.1 mmHg S.D.). The heart rate and blood pressure changes elicited by isoproterenol infusion disappeared rapidly after infusion was discontinued whereas up to 20 min were required for the chemical composition of the blood to return to preinfusion values.

DISCUSSION

Intravenous infusion of isoproterenol into pregnant rhesus monkeys consistently elicited a decrease in oxygen tension of the maternal arterial blood. Review of the literature failed to uncover any information regarding such an effect on arterial pO_2 during pregnancy in any species. The few clinical reports that cite any alterations in oxygenation during infusion or inhalation of beta adrenergic drugs describe small reductions in arterial blood pO_2 in asthmatic patients while failing to demonstrate such changes in controls (9, 10). The authors of these studies attribute the reductions in arterial blood pO_2 in asthmatic patients to an increased maldistribution of pulmonary ventilation due to uneven penetrance of the aerosol within the tracheobronchial tree. Because in the present study isoproterenol was administered intravenously and hence could not affect the terminal air ducts nonhomogeneously the significant decreases in arterial blood pO_2 observed are more likely due to an increased right to left shunting within the pulmonary vasculature. This interpretation is consistent with the finding that infusion of isoproterenol increases the conductance of the pulmonary blood vessels and augments the

blood volume of the capillary bed in isolated lung preparations (11).

The rapid injection of isoproterenol in the mothers consistently increased the heart rate and widened the pulse pressure of the well-oxygenated fetuses. These changes in cardiovascular function of the fetuses followed similar changes observed in the mothers by an interval of about 40 sec and persisted for a similar period of time. Increase in maternal heart rate accompanied by a similar increase in the fetus has also been observed recently in man in conjunction with administration of beta-adrenergic agents (8, 11). Because the fetus regularly decreased following administration of isoproterenol to the mother the increase in fetal heart rate and pulse pressure can be attributed solely to an effect of isoproterenol acting directly on the fetus following its transfer from the mother.

When isoproterenol was administered to the mothers of poorly oxygenated fetuses bradycardia and hypotension of the fetuses was observed. The differences in cardiovascular response of the fetuses according to their preexisting pO_2 values is explained by the fact that a relatively large reduction in arterial blood pO_2 is required to elicit fetal bradycardia and hypotension in the well-oxygenated fetus. Lowering of pO_2 of small magnitude did not occur during the infusion of isoproterenol to the mothers of well-oxygenated fetuses.

Beta adrenergic receptor stimulating agents do not reduce oxygenation of the fetus through direct mechanisms. The first is the reduction in maternal arterial pO_2 presumably due to increased right to left shunting. The second consists of reduction of uterine or intervillous space blood flow by increasing the conductance of blood vessels supplying organs which are rich in beta adrenergic receptors. Such changes are more likely to occur with beta adrenergic agents which have little positive chronotropic and inotropic effect and hence do not increase appreciably myocardial output. It should also be recognized that maintenance of mean arterial blood pressure during administration of the agent does not ensure maintenance of a given flow rate to the uterus or the intervillous space. This is due to the fact that reflexly mediated regional vasoconstriction with resultant reduction in conductance is not linked specifically to changes in mean arterial pressure.

The third mechanism depends on a direct effect

isoproterenol on fetal vasculature diverting the ventricular output of the fetus from the perfusion of the placenta

The relative contribution made by these mechanisms will depend on the status of uterine blood flow, the time of administration of the agent relative to uterine contractions, the degree of entrance of the drug into the fetal compartment, reactivity and vascular status of other areas of maternal vascular tree and the bioavailability of the compound.

Although an extensive literature depicts the cardiovascular effects of infusion of isoproterenol in other related compounds in a variety of species in the nonpregnant state, little information is available regarding its effect on cardiac output and regional blood flow distribution during pregnancy. In pregnant ewes infusion of isoproterenol has been reported to increase slightly uterine blood flow and to cause a decrease in perfusion pressure (12). This finding has been interpreted as indicating that uterine circulation is under the regulatory control of beta adrenergic receptors. More recently similar results have been obtained in studies with synthetic beta adrenergic receptor stimulating agents including isoxsuprine and nitroglycerin where infusions into awake pregnant ewes have led to greater than 30% reductions in uterine blood flow. The present data favor the contention that the responsiveness of uterine blood vessels, including spiral arteries, to beta adrenergic stimulants is greater than that of blood vessels elsewhere in the body and that their administration leads to a decrease rather than an increase in perfusion of the intervillous space.

The hypoxic and acidotic fetus was particularly sensitive to changes in circulation in the mother brought about by administration of isoproterenol. It should be emphasized, however, that it is the low tolerance of the asphyxiated fetus to further decrease in oxygenation that accounts for this response rather than any specific sensitivity of animals to the pharmacologic agent. The effects of isoproterenol upon pO_2 of fetal blood as reported in the present study are similar to those inferred from clinical experience with other beta mimetic agents used to control excessive uterine activity. Caldeyro-Barcia and colleagues (5) obtained evidence of an amelioration of fetal condition as judged by improvement of fetal heart rate patterns following administration of

isoprenaline to the mother in hyperactive labor. Saling and his collaborators have also provided data taken from a larger patient series that describe a definite value in administering beta mimetic agents for the treatment of fetal asphyxia under such circumstances (14).

Several factors may account for these apparent discrepancies in outcome. In our studies isoproterenol was administered to pregnant monkeys exhibiting only slight uterine activity in contrast to the above described clinical studies where corresponding agents were employed only in the presence of a uterine activity so great as to cause a clinically significant asphyxia of the fetus. Reduction of uterine activity under such conditions is likely to lead to a net increase in intervillous space blood flow in spite of the increase in vascular conductance of blood vessels of other organs. Other factors that may contribute to differences in outcome include differences in sedation or anesthesia of the mother and fetus, differences in pharmacologic properties of the various betamimetic drugs used and differences in reactivity of the uterus and cardiovascular system among different species of primates.

Isoproterenol administered directly to the fetus causes significant reductions in oxygen pressure of fetal aortic blood without inducing any changes in heart rate or blood pressure of the mother. This direct drug effect on the fetus likely results from an increased conductance of the blood vessels supplying the lungs and other tissues of the fetus, diverting blood flow from the umbilical circulation. This interpretation is favored by the fact that infusion of isoproterenol directly into the sheep fetus markedly increases blood flow through the lungs (15).

ACKNOWLEDGMENTS

The authors express their appreciation to Mr Esteban Monell Torrens for his assistance in carrying out this study.

REFERENCES

1. Liggins G C & Vaughan G N. *J Obstet Gynaecol Br Comm* 80: 29, 1973.
2. Castrén O, Gummerus M & Saarikoski S. *Acta Obstet Gynecol Scand* 54: 95, 1975.
3. Wesselius De Caspans A, Thierry M, You L, Sian A, Baumgarten K, Brosens J, Gamusans O, Stolk J G & Vivier W. *Br Med J* 3: 144, 1971.
4. Renaud R, Irrmann M, Gandar R & Flynn M D. *J Obstet Gynaecol Br Comm* 81: 182, 1974.

- 5 Caldeyro Barcia R, Magana J M, Castillo J B, Poseiro J J, Mendez Bauer C, Pose S V, Escarcena I, Casacuberta C, Bustos J R & Giusi G. In Perinatal Factors Affecting Human Development. Pan American Health Organization Sci Publ No 183 Washington DC 1969 pp 248-253
- 6 Lauenstein N H, Wilson K H & Fuchs F. Am J Obstet Gynecol 121: 597 1975
- 7 Adamsons K, Mueller Heubach F & Myers R E. Am J Obstet Gynecol 109: 248 1971
- 8 Miller F C, Nochimson D J, Paul R H & Hon E H. Obstet Gynecol 47: 50 1976
- 9 Knudson R J & Constantine H P. J Appl Physiol 23: 403 1967
- 10 Chick T W, Nicholson D P & Johnson R L. Am Rev Resp Dis 107: 869 1973
- 11 Brody J S & Stemmler E J. J Clin Invest 47: 800 1968
- 12 Ladner C N, Brinkman C R, Weston P & Luce N J. Am J Physiol 218: 257 1970
- 13 Chez H A, Ehrenkranz R A, Oakes G K, Walker A M, Hamilton L A, Brennon S C & McLaughlin M K. In Fetal and Neonatal Nutrition (ed L D Longo and D D Renshaw). Ciba STPM Press New York
- 14 Sahing E. Personal communication
- 15 Dawes G S. Foetal and Neonatal Physiology pp 79-90. Year Book Medical Publishers Chicago 1962

Submitted for publication Febr 15 1977

Ingemar Joelsson M D
Department of Obstetrics and Gynecology
Umeå University Hospital
S 90185 Umeå
Sweden

EFFECTS OF TERBUTALINE ON THE PRESSURE VOLUME RELATIONSHIP IN FETAL RABBIT LUNG

B Bergman T Hedner and P Lundborg

From the Department of Obstetrics and Gynaecology Centrallasarettet Mölndal and the Department of Pharmacology University of Göteborg Göteborg Sweden

Abstract The pressure volume relationship in preterm fetal lung was studied at 28 days of gestation. Injection of 0.1 mg terbutaline, a selective β_2 -receptor stimulating agent, significantly increased the volume of air at equivalent low transpulmonary pressures compared to a saline treated group and an untreated group. These findings indicate an increased pulmonary distensibility of the fetal rabbit lung after terbutaline administration. The mechanism of action is discussed and surfactant mediated changes are suggested to be the probable explanation.

Perinatal respiratory distress syndrome (IRDS) or hyaline membrane disease (HMD) is a common serious condition occurring mainly in preterm infants. Clinical studies during recent years have indicated that the risk of HMD can be reduced by administration of various drugs to the mother during pregnancy.

The possibility that corticosteroids present in fetal circulation may influence pulmonary maturation was reported by Buckingham (3). Liggins and Liggins & Howie (13). Other investigators have had a similar effect by isoxsuprine (8, 17), dexamethasone (7), heroin (6) and by thyroxine (14, 16). In a clinical study we have administered terbutaline, a selective β_2 -receptor stimulating drug, during pregnancy in an attempt to arrest premature labour where delivery occurred in spite of this treatment. We found a remarkably low incidence of IRDS as compared to a control group which had received no such treatment (1).

The aim of the present investigation was to examine the potential usefulness of terbutaline in this respect by studying its effect on the fetal rabbit lung under experimental conditions.

Copulation was considered as zero time of gestation. The does were kept in separate cages in the department and fed on standard pellets and water ad libitum.

Three days before term at 28 days of gestation the pregnant does were anesthetized with ether administered by open mask and a laparotomy was performed to expose the uterus. Normal saline (0.1 ml) or terbutaline (0.1 mg in 0.1 ml normal saline) was injected intramuscularly into the fetuses through the intact uterine wall. After this procedure the laparotomy incision was closed. The whole operation never took more than 10 min. The animals recovered and 3 h later the does were again anesthetized with ether and the uterus exposed through the same incision. In another control group the does were operated only once and no fetuses received any injections.

In all cases a ligature was tied around the upper part of the vagina and the intact uterus was removed and immersed in normal saline at room temperature. Immediately after delivery of the fetuses a ligature was tied around the umbilical vessels and the neck to prevent spontaneous breathing.

The litter size varied between 5 and 13 and the fetal weights between 35.0 and 53.5 g.

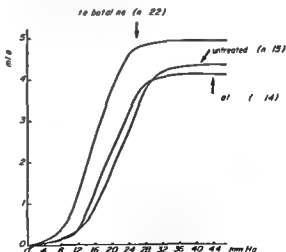


Fig 1 Inflation pressure volume curves in fetal rabbit lung at 28 days of gestation. Comparison between terbutaline treated, saline treated and untreated.

MATERIAL AND METHODS

Female and black rabbits of Danish rural breed were used, one or two bucks under direct observation.

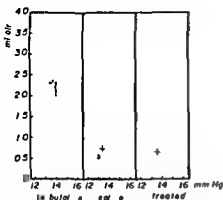


Fig 2 Pressure volume relationships in inflation of air in fetal rabbit lung at 78 days of gestation. Comparison between terbutaline treated, saline treated and untreated animals in the pressure interval 12.0–15.9 mmHg.

Excluded were three does with premature spontaneous delivery before the second laparotomy. Also excluded were fetuses where the period between the second laparotomy and the experimental procedure exceeded 3 h and fetuses where technical errors occurred during preparation. The trachea of the fetuses was exposed and a cannula was inserted. The cannula was connected to a syringe and a pressure transducer (Statham P23 BC) in an air tight system and pressure curves were recorded by a Grass Polygraph (model 7). Pressure volume curves of the lungs were established using inflation with air into the intact fetuses. Pressure readings at different levels were registered after one minute of equilibration and maximum pressures were noted at the bursting of the lungs. For each of the fetuses 5–10 steps of inflation were made. Recording was performed in all animals within 3 h after death. An interval reported not to change the pressure volume curves (11).

Statistical evaluation was carried out by Student's *t* test and Fisher's permutation test followed by the Edgeworth distribution. *P* values less than 0.05 were considered as statistically significant.

RESULTS

The mean inflation pressure curves at 28 days of gestation for 22 terbutaline treated, 14 saline treated and 15 untreated animals are shown in Fig 1. The curves of the saline and untreated groups are approximately equal. In the terbutaline group there is a shift to the left and a larger maximum volume is achieved.

The pressure volume relationships in different pressure intervals are presented in Fig 2 and Table 1. Significantly higher volume recordings were noted in the terbutaline group compared to the two control groups except in the interval 28.0–31.9 mmHg where the difference was not statistically significant compared to the untreated group.

Table 1 Pressure volume relationship in different pressure intervals in fetal rabbit lung

Mean \pm S.E.M.

Pressure interval	Terbutaline	Saline	Untreated
4.0–7.9 mmHg			
mmHg	5.71 \pm 0.23	6.12 \pm 0.18	6.04 \pm 0.17
ml air	0.17 \pm 0.07	0.10 \pm 0.01	0.17 \pm 0.03
n	19	14	15
8.0–11.9 mmHg			
mmHg	9.64 \pm 0.14	10.18 \pm 0.14	10.17 \pm 0.12
ml air	0.58 \pm 0.07	0.23 \pm 0.01	0.23 \pm 0.01
n	27	9	17
12.0–15.9 mmHg			
mmHg	13.74 \pm 0.16	13.11 \pm 0.10	13.60 \pm 0.13
ml air	2.01 \pm 0.15	0.64 \pm 0.04	0.64 \pm 0.11
n	27	14	15
16.0–19.9 mmHg			
mmHg	17.50 \pm 0.0	17.58 \pm 0.34	18.17 \pm 0.8
ml air	3.57 \pm 0.18	1.88 \pm 0.04	1.57 \pm 0.8
n	18	13	13
20.0–23.9 mmHg			
mmHg	21.08 \pm 0.17	21.70 \pm 0.34	21.67 \pm 0.8
ml air	3.48 \pm 0.16	2.79 \pm 0.15	2.19 \pm 0.8
n	10	10	10
24.0–27.9 mmHg			
mmHg	25.70 \pm 0.49	25.77 \pm 0.13	25.71 \pm 0.8
ml air	4.77 \pm 0.31	3.26 \pm 0.16	3.31 \pm 0.8
n	5	7	12
28.0–31.9 mmHg			
mmHg	30.90 \pm 0.14	29.25 \pm 0.15	29.25 \pm 0.8
ml air	4.77 \pm 0.15	3.40 \pm 0.40	3.77 \pm 0.8
n	5	2	4
32.0–40.0 mmHg			
mmHg	36.05 \pm 0.79	36.45 \pm 0.80	38.00 \pm 1.1
ml air	4.88 \pm 0.13	3.69 \pm 0.19	3.74 \pm 0.8
n	11	11	5

Significance indicated versus the terbutaline group: $p < 0.05$, $p < 0.01$, $p < 0.005$, $p < 0.001$. Versus the saline group: $p < 0.05$, $p < 0.01$.

The mean transpulmonary pressures in the different pressure intervals were found to be equal or significantly lower in the terbutaline group compared to the control groups except in the intervals 12.0–15.9 mmHg and 28.0–31.9 mmHg where the mean pressures were slightly lower in the control groups. To adjust for this difference in mean pressures in statistical evaluation of inflated volumes, observations of the terbutaline and saline groups in the interval 12.0–15.9 mmHg were divided into two groups and analyzed by Fisher's permutation test. Following this correction a significantly greater volume was found in the terbutaline group in the interval 28.0–31.9 mmHg. No such evaluation could

ade because of the small number of observa
 comparing the two control groups a significantly
 er volume was found in the saline group in the
 vals 16.0–19.9 mmHg and 20.0–23.9 mmHg
 such the mean pressures recorded in these in
 als were equal. In all other intervals no signifi
 differences were found in volume or pressure
 een the two control groups
 ve mean fetal weight in the terbutaline group
 43.7 ± 1.2 g (mean ± S.E.M.) in the saline
 g 47.6 ± 1.5 g and in the untreated group
 ± 1.0 g. No significant differences were found
 een the mean weights of the groups

DISCUSSION

he last part of gestation the mammalian lung
 goes rapid maturation. During this period in
 sed amounts of elastic tissue and alveolar
 tures appear in the fetal lung. Parallel to this
 lopment there is an increased formation of
 antant from type II cells of the alveolar lining.
 In 1969 Liggins observed that lambs delivered
 prenatal glucocorticosteroid administration
 viable before pulmonary surfactant would
 ally be present in amounts sufficient to
 ilize the alveoli. These results suggested that
 maturation in some way had been accelerated.
 Other investigators showed that administra
 of corticosteroids into rabbit fetuses two to
 e days before preterm delivery resulted in an
 lerated appearance of alveolar surfactant (9).
 Similar results have also been reported in in
 experiments (15). An increased lung volume at
 transpulmonary pressure in fetal rabbit has also
 demonstrated after isoxuprine administration
 e days before term. The phenomenon was
 already 3.5 h after injection of the drug (17).
 authors suggest that the mechanism of this
 action might be an enhanced release of

rabbit fetuses pulmonary surfactants are de
 le at day 24 and increase markedly as preg
) proceeds (7–10). At 28 days of gestation the
 opical maturation in all parts of the fetal
 lung is known to be completed (10). Previous
 ave shown that the volume of air present at
 transpulmonary pressures correlates well to
 parameters of measuring alveolar surface ac
 1 (5).

In our experiments we have used a β_2 receptor
 stimulating drug terbutaline with known selective
 bronchodilating and uterine relaxing properties in
 jected at day 28 of gestation into rabbit fetuses. An
 increased air volume capacity was found in the
 terbutaline treated animals at equivalent low trans
 pulmonary pressures in all intervals as compared to
 the controls. Interesting to note was also the pres
 ence of a significantly higher volume at equivalent
 pressures in the interval 32.0–39.9 mmHg where
 several of the maximum pressures occurred indi
 cating an increased total lung capacity after
 terbutaline treatment.

Comparison between the two control groups re
 vealed a significantly higher volume in the saline
 treated group at equivalent mean pressure in the
 intervals 16.0–19.9 mmHg and 20.0–23.9 mmHg. As
 differences in lung volume were noted only in these
 two intervals it is doubtful if a stress factor like a
 second laparotomy has any effect on the pulmo
 nary distensibility in fetal rabbit lung.

Although surfactant mediated effects probably
 account for the shift in the pressure volume rela
 tionship after β mimetic stimulation there are also
 other possible mechanisms of action as alteration in
 tissue elasticity or relaxation of the bronchial
 smooth muscle cells.

Our experimental evidence should therefore be
 supplemented with a direct determination of the
 amount and properties of the surface active material
 in the alveolar lining layer.

ACKNOWLEDGEMENTS

This research was supported by the Swedish State Medi
 cal Research Council (No. 2464) and Expressens Pre
 natalforskningsfond. The reading and revising of the man
 uscript by Professor N. Wijkström and Assistant Professor I.
 Kjellmer is gratefully acknowledged. The authors also
 wish to thank AB Draco, Lund, Sweden for generously
 supplying terbutaline sulfate.

REFERENCES

1. Bergman H & Hedner T. Antepartum administra
 tion of terbutaline and the incidence of hyaline
 membrane disease in preterm infants. *Acta Obstet
 Gynecol Scand*. In press.
2. Boog G, Ben Brahim M & Gandar M. Beta
 mimetic drugs and possible prevention of respiratory
 distress syndrome. *Br J Obstet Gynecol* 82: 785, 1975.
3. Buckingham S, McNary W F, Sommers S C &
 Rothschild J. Is lung an analog of Moog's developing

- intestine? I Phosphatases and pulmonary alveolar differentiation in fetal rabbits *Fed Proc* 27: 378 1968
- 4 Charnock E L & Doershuk C F Developmental aspects of the human lung *Pediatr Clin North Am* 20: 275 1973
 - 5 Clements J A Hustead R F Johnson R P & Gribets I Pulmonary surface tension and alveolar stability *J Appl Physiol* 16: 444 1961
 - 6 Glass L Rajegoweda B & Evans H Absence of respiratory distress syndrome in premature infants of heroin addicted mothers *Lancet* 2: 685 1971
 - 7 Gluck L Stribney M & Kulovich M V The biochemical development of surface activity in mammalian lung II The biosynthesis of phospholipids in the lung of the developing rabbit fetus and newborn *Pediatr Res* 1: 247 1967
 - 8 Kero P Hirvonen T & Valimäki I Prenatal and postnatal isoxuprine and respiratory distress syndrome *Lancet* 2: 199 1973
 - 9 Kikkawa Y Kaibara M Motoyama E K Orzalessi M M & Cook C D Morphologic development of fetal rabbit lung and its acceleration with cortisol *Am J Pathol* 64: 423 1971
 - 10 Kikkawa Y Motoyama E K & Gluck L Study of the lungs of fetal and newborn rabbits Morphologic, biochemical and surface physical development *Am J Pathol* 52: 177 1968
 - 11 Kotas R V & Avery M E Accelerated appearance of pulmonary surfactants in the fetal rabbit *J Appl Physiol* 30: 358 1971
 - 12 Liggins G C Premature delivery of fetal lambs infused with glucocorticoids *J Endocrinol* 45: 115 1969
 - 13 Liggins G C & Howie R N A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants *Pediatrics* 50: 515 1972
 - 14 Redding H A Douglas W H & Stein M Thyroid hormone influence upon the lung surfactant metabolism *Science* 175: 994 1971
 - 15 Smith H T & Torday J S Factors affecting histidine synthesis by fetal lung cells in culture *Pediatr Res* 8: 848 1974
 - 16 Wu B Kikkawa Y Orzalessi M M Motoyama E K Kaibara M Zigas C J & Cook C D Accelerated maturation of fetal rabbit lungs by thymine *Physiol* 14: 253 1974
 - 17 Wyszogrodski I Taeusch H W & Avery M E Isoxuprine induced alterations of pulmonary pressure volume relationship in premature *Am J Obstet Gynecol* 119: 1107 1974

Submitted for publication March 10 1977

Thomas Hedner
Department of Pharmacology
Fack
S-40033 Göteborg 33
Sweden

THE EFFECTS OF LONGACTING PARACERVICAL BLOCK ANESTHESIA ON THE ABORTIFACIENT EFFICACY OF INTRA AMNIOTIC PGF₂ AND HYPERTONIC SALINE

M I Ragab¹ D A Edelman and L. Laue

From the ¹Ain Shams University, Cairo, Egypt and the ²International Fertility Research Program, Research Triangle Park, N C, USA

Abstract A comparative study was conducted to evaluate effects of repeated longacting paracervical blocks on abortifacient efficacy of intraamniotic prostaglandin PGF₂ 40 mg initially and an additional 20 mg after 4 hours—and hypertonic saline augmented with intravenous oxytocin for patients at 16 to 20 weeks gestation. Patients were randomly assigned to the 2 abortion procedures and one half (50) of the patients induced with procedure received serial longacting paracervical blocks. For those patients aborted with saline the rates of side effects incomplete abortion and relative abortion were similar for patients whether they did or did not receive paracervical blocks. Among the PGF₂-treated patients who were administered paracervical blocks there was a significant reduction in the rates of gastrointestinal side effects and incomplete abortion and a significant difference in the cumulative abortion rates in 37 hours of the initial PGF₂ instillation 98% of the patients who received paracervical blocks aborted compared to 70% of those who did not receive paracervical blocks. Although the cumulative abortion rates of PGF₂-treated patients with paracervical blocks and PGF₂-treated patients were similar the rate of incomplete abortion for the PGF₂ treated patients was significantly lower. Additional studies will be necessary to evaluate the efficacy and advantages of using paracervical block anesthesia as an adjunct to midtrimester abortion procedures.

It is recently when prostaglandin F₂ (PGF₂) became commercially available for intra amniotic use

the instillation of hypertonic saline augmented with intravenous oxytocin was the most widely used procedure for midtrimester abortion. Several studies (1, 3, 4, 5) have indicated that effective intra amniotic PGF₂ dose schedules may be safer than the intra amniotic administration of hypertonic saline with or without oxytocin augmentation. However vomiting, diarrhea and pelvic pain are more frequent with effective PGF₂ methods (2). The administration of antiemetics and antidiarrheal agents may attenuate vomiting and diarrhea. A recent report from CDC challenges the concept that saline produces a higher complication rate (8). One must remember that all cases in this study were from the U S A. Racial characteristics may contribute to the variations in results generated in international trials (10).

This study was conducted to test the hypothesis that the administration of repeated longacting paracervical blocks from the time of the initial instillation up to the time the fetus is aborted may result in shortened instillation-to-abortion times in addition to reducing the discomforts of the abortion process of the following 2 intra amniotic dose schedules: (1) 40 mg PGF₂ initially and an addi-

Table 1 Side effects and complications of PGF₂ and saline with and without paracervical block anesthesia

Effect/complication	PGF ₂		Saline	
	No PCB (N=49) (%)	With PCB (N=50) (%)	No PCB (N=50) (%)	With PCB (N=49) (%)
1st	24.5	8.0	0.0	0.0
2nd	28.6	12.0	0.0	0.0
3rd	6.1	0.0	0.0	0.0
4th	0.0	0.0	1.0	8.2
>38°C	4.1	0.0	4.0	0.0
laceration	0.0	0.0	0	2.0

- intestine? I Phosphatases and pulmonary alveolar differentiation in fetal rabbits *Fed Proc* 27 328 1968
- 4 Charnock E L & Doershuk C F Developmental aspects of the human lung *Pediatr Clin North Am* 20 275 1973
- 5 Clements J A Hustead R F Johnson R H & Gribets I Pulmonary surface tension and alveolar stability *J Appl Physiol* 16 444 1961
- 6 Glass L Rajagoweda B & Evans H Absence of respiratory distress syndrome in premature infants of heroin addicted mothers *Lancet* 2 685 1971
- 7 Gluck L Sribney M & Kulovich M V The biochemical development of surface activity in mammalian lung II The biosynthesis of phospholipids in the lung of the developing rabbit fetus and newborn *Pediatr Res* 1 247 1967
- 8 Kero P Hirvonen T & Valimäki I Prenatal and postnatal isoxuprine and respiratory distress syndrome *Lancet* 2 198 1973
- 9 Kikkawa Y Kaibara M Motoyama E K Orzalesi M M & Cook C D Morphologic development of fetal rabbit lung and its acceleration with cortisol *Am J Pathol* 64 423 1971
- 10 Kikkawa Y Motoyama E K & Gluck L Study of the lungs of fetal and newborn rabbits Morphologic biochemical and surface physical development *Am J Pathol* 52 177 1968
- 11 Kotas R V & Avery M E Accelerated appearance of pulmonary surfactants in the fetal rabbit *J Appl Physiol* 30 358 1971
- 12 Liggins G C Premature delivery of fetal lambs infused with glucocorticoids *J Endocrinol* 41 45 1969
- 13 Liggins G C & Howie R N A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants *Pediatrics* 50 515 1972
- 14 Redding R A Douglas W H & Stein M Thyroid hormone influence upon the lung surfactant metabolism *Science* 175 994 1972
- 15 Smith B T & Torday J S Factors affecting keratin synthesis by fetal lung cells in culture *P.L. Res* 8 848 1974
- 16 Wu B Kikkawa Y Orzalesi M M Motoyama E K Kaibara M Zigas C J & Cook C D Accelerated maturation of fetal rabbit lungs by thyroxine *Physiologist* 14 253 1974
- 17 Wyszogrodski J Taeusch H W & Avery M E Isoxuprine induced alterations of pressure volume relationship in premature *Am J Obstet Gynecol* 119 1107 1974

Submitted for publication March 10 1977

Thomas Hedner
Department of Pharmacology
Fack
S-40033 Göteborg 33
Sweden

THE EFFECTS OF LONGACTING PARACERVICAL BLOCK ANESTHESIA ON THE ABORTIFACIENT EFFICACY OF INTRA AMNIOTIC PGF₂ AND HYPERTONIC SALINE

M. I. Ragab¹, D. A. Edelman and L. Laufe

From the ¹Ain Shams University, Cairo, Egypt and the ²International Fertility Research Program, Research Triangle Park, N. C., USA

A comparative study was conducted to evaluate the effects of repeated longacting paracervical blocks on the abortifacient efficacy of intraamniotic prostaglandin (PGF₂)—40 mg initially and an additional 40 mg after 6 hours—and hypertonic saline augmented with intravenous oxytocin for patients at 16 to 20 weeks gestation. Patients were randomly assigned to the 2 abortion procedures and one half (50) of the patients induced with the procedure received serial longacting paracervical blocks. For those patients aborted with saline, the rates of complications and side effects, incomplete abortion and repeat abortion were similar for patients whether they did or did not receive paracervical blocks. Among the PGF₂ treated patients who were administered paracervical blocks, there was a significant reduction in the rates of gastrointestinal side effects and incomplete abortion and a significant difference in the cumulative abortion rates. In 33 hours of the initial PGF₂ instillation 98% of the patients who received paracervical blocks aborted compared to 70% of those who did not receive paracervical blocks. Although the cumulative abortion rates of the treated patients with paracervical blocks and the untreated patients were similar, the rate of incomplete abortion for the PGF₂ treated patients was significantly lower. Additional studies will be necessary to evaluate the benefits and advantages of using paracervical block anesthesia as an adjunct to midtrimester abortion procedures.

Recently when prostaglandin F₂ (PGF₂) became commercially available for intra amniotic use

the instillation of hypertonic saline augmented with intravenous oxytocin was the most widely used procedure for midtrimester abortion. Several studies (1, 3, 4, 5) have indicated that effective intra amniotic PGF₂ dose schedules may be safer than the intra amniotic administration of hypertonic saline with or without oxytocin augmentation. However, vomiting, diarrhea and pelvic pain are more frequent with effective PGF₂ methods (2). The administration of antiemetics and antidiarrheal agents may attenuate vomiting and diarrhea. A recent report from CDC challenges the concept that saline produces a higher complication rate (8). One must remember that all cases in this study were from the U.S.A. Racial characteristics may contribute to the variations in results generated in international trials (10).

This study was conducted to test the hypothesis that the administration of repeated longacting paracervical blocks from the time of the initial instillation up to the time the fetus is aborted may result in shortened instillation-to-abortion times in addition to reducing the discomforts of the abortion process of the following 2 intra amniotic dose schedules: (1) 40 mg PGF₂ initially and an addi-

1 Side effects and complications of PGF₂ and saline with and without paracervical block anesthesia

Complication	PGF ₂		Saline	
	No PCB (N=49) (%)	With PCB (N=50) (%)	No PCB (N=50) (%)	With PCB (N=49) (%)
nausea	24.5	8.0	0.0	0.0
	28.6	1.0	0.0	0.0
	6.1	0.0	0.0	0.0
>38°C	0.0	0.0	1.0	8.2
diarrhea	4.1	0.0	4.0	0.0
	0.0	0.0	2.0	0.0

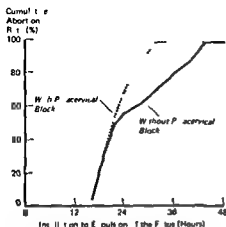


Fig 1 Cumulative abortion rates for the 40-20 mg intra amniotic prostaglandin F_2 dose schedule with and without paracervical block anesthesia

tional 20 mg PGF_2 after 24 hours if abortion had not yet occurred and (2) 200 ml of 20% hypertonic saline augmented with intravenous oxytocin

METHODS AND MATERIALS

From April to September 1976 a comparative study was conducted to assess the effects of serial longacting paracervical block anesthesia on the abortifacient efficacy of intra amniotic $PGF_{2\alpha}$ and hypertonic saline augmented with intravenous oxytocin for aborting patients at 16 to 20 weeks gestation

Abortion procedures

Patients were aborted with 1 of 2 intra amniotic techniques (1) an initial dose of 40 mg PGF_2 with an additional 20 mg after 24 hours (100 patients) and (2) 200 ml of 20% hypertonic saline without prior aspiration of the amniotic fluid augmented with low doses of intravenous oxytocin (100 patients). One half of the patients aborted by each of the above techniques received longacting paracervical blocks starting at 4 hours after the time of the initial drug instillation and repeated every 6 hours (up to a maximum of four injections) until abortion of the fetus. Paracervical blocks were not given if the cervix was effaced at the scheduled time of injection. The paracervical blocks were performed by injecting 5 cc of 1% lidocaine with epinephrine 1:100,000 at the 4 and 8 o'clock positions.

For all patients the abdominal wall was sterilized and anesthetized with 1% lidocaine and the uterus was punctured with an 18 gauge needle. A small amount of amniotic fluid was withdrawn to verify correct placement of the needle. Prostaglandin was administered through a polyethylene catheter threaded through the needle. A test dose of 5 mg PGF_2 was administered over a 5 min period and an additional 35 mg was injected over the next 5 min if there were no adverse reactions. An additional 20 mg PGF_2 was administered if abortion did not occur within 24 hours unless there was uterine bleeding, ruptured membranes or cervical effacement or dilation.

A maximum of 200 ml 20% hypertonic saline slowly instilled through a transabdominally placed catheter over a period of at least 5 min. If during the 20 min syringe was being refilled there was a marked reflux of fluid from the catheter no additional saline was administered. The mean amount of saline instilled was 150 ml the range was from 140 to 200 ml.

Following instillation all patients received only 1 meperidine hydrochloride intramuscularly.

Two hours after the initial PGF_2 instillation, patients received an IV drip of a 5% dextrose solution at 100 ml/minute. At the onset of labor pains patients with hypertonic saline were administered a constant intravenous infusion of oxytocin in a 5% dextrose solution which was regulated according to uterine response on a Harvard infusion pump. The rate of the infusion inversely with the strength, duration and frequency of uterine contractions and with the condition of the cervix. The infusion rates varied from 0 to 110 mU/min (54 mU/min) and reflected the differing physiologic responses of the patients to the oxytocin. After rupture of the membranes and/or abortion of the fetus oxytocin was infused at a rate of about 100 mU/min.

Subjects

Only healthy women without preexisting systemic or gynecologic diseases were selected as subjects for the study. Patients aborted with either of the study procedures were similar ($p > 0.10$) with respect to their ductive histories and other selected characteristics. Mean age was 33.1 years (range 22 to 44) and the parity was 3.9 live births (range 0 to 7). Four percent nulliparas, 34.5% were grandmultiparas and 61.5% reported having a previous spontaneous or induced abortion.

Study design

The abortion procedures (intra amniotic PGI_2 hypertonic saline with or without paracervical block anesthesia) were randomly assigned to subjects following manner. Before initiating the abortion the physician responsible for performing the pro-

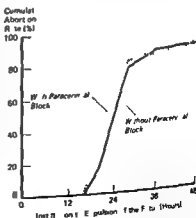


Fig 2 Cumulative abortion rate for intra amniotic with and without paracervical block anesthesia

Table II Median times (hours) from instillation to onset of labor, uterine bleeding, rupture of the membranes and abortion of the fetus for PGF₂ and saline with and without paracervical block anesthesia

	PGF ₂		Saline	
	No PCB (N=49)	With PCB (N=50)	No PCB (N=50)	With PCB (N=49)
Instillation to labor	2.1	2.3	7.4	11.6
Instillation to uterine bleeding	17.3	13.8	16.1	14.6
Instillation to rupture of membranes	21.3	19.8	21.5	19.4
Instillation to abortion	22.1	21.3	24.0	22.7

ed a sealed envelope which specified the particular procedure for the patient. A second physician, who was unable for follow-up care and evaluation of the patient, was kept unaware of the particular procedure used by patient.

Patients were requested to return for a follow-up physical examination at about four weeks after the abortion. Follow-up data were obtained for 97.5% of the patients.

Statistical methods and criteria

Gestational age was calculated as the number of completed weeks from the onset of the patient's last normal menstrual period to the day of the abortion. If the patient failed to abort the fetus within 48 hours of the initial instillation, the trial was declared a failure and other appropriate therapy was initiated. The abortion was declared incomplete when the fetus delivered without assistance but intervention was required to deliver the placenta. If the patient failed to completely expel the placenta within one hour of aborting the fetus, the placenta was removed with ring forceps. The level of significance level of statistical tests (the α level) is given.

RESULTS

Amniocentesis could not be performed even after repeated attempts in 2 patients at 16 and at 18 weeks gestation. They underwent hysterotomy with concurrent tubal ligation and were excluded from analyses.

Among patients aborted with PGF₂, rates of side effects were significantly lower ($p < 0.10$) for those with longacting paracervical blocks than for those without them (Table I). Between the 2 groups of patients aborted with saline, there was no significant differences ($p > 0.10$) in the rates of side effects or complications (Table I). Two patients aborted with saline had cervical lacerations, neither of which required repair. Blood loss was less than 300 ml for all patients, and no patient required blood replacement therapy.

While the cumulative abortion rates were not significantly different ($p > 0.10$) for the 2 groups of patients administered saline (Fig. 1), they were significantly different ($p < 0.10$) for patients administered PGF₂ with or without paracervical block anesthesia (Fig. 2). The cumulative abortion rate for PGF₂ with paracervical block anesthesia was consistently higher than the cumulative rate for saline with or without paracervical block anesthesia, but the cumulative rates were not significantly different ($p > 0.10$).

The median times from instillation of PGF₂ or saline to the onset of uterine bleeding, rupture of the membranes, and abortion were not significantly different ($p > 0.10$) (Table II). Although the median

Table III Rates of incomplete abortion with PGF₂ and saline with and without paracervical block anesthesia (PCB)

Outcome	PGF ₂		Saline	
	No PCB (N=49) (%)	With PCB (N=50) (%)	No PCB (N=50) (%)	With PCB (N=49) (%)
Retention of fetus and placenta	42.9	78.0	40.0	51.0
of placenta with ring forceps	55.1	20.0	50.0	40.8
within 48 hours	7.0	7.0	10.0	8.7

times from instillation to the onset of labor were not significantly affected by the paracervical blocks; they were significantly lower ($p < 0.10$) for patients aborted with $\text{PGF}_{2\alpha}$ compared to those aborted with saline (Table II).

An additional 20 mg of $\text{PGF}_{2\alpha}$ was more often given to patients who did not receive paracervical blocks (38.8%) than to those who did (12.0%). Two patients administered $\text{PGF}_{2\alpha}$ failed to abort within the 48 hour trial period (Table III). These patients were administered intravenous oxytocin (100 mIU/min) and aborted after 72.2 and 74.2 hours. Nine (91%) patients administered saline failed to abort within the 48 hour trial period. All of these patients had signs of impending abortion (uterine bleeding) and aborted from 48.7 to 68.2 hours after the initial saline instillation.

Rates of complete abortion were significantly higher ($p < 0.10$) for the $\text{PGF}_{2\alpha}$ treated subjects with paracervical block anesthesia than without paracervical block anesthesia (Table III). However, for the 2 groups of patients administered saline, the rates of complete and incomplete abortion were not significantly different ($p > 0.10$).

For patients who did not receive paracervical blocks, meperidine (50 mg IM) was more often needed for the comfort of the patient at the time of placenta removal ($\text{PGF}_{2\alpha}$ without paracervical block 51.9% with paracervical block 20.0% saline without paracervical block 28.0% with paracervical block 0.0%).

DISCUSSION

Although the administration of longacting paracervical block anesthesia significantly improved the abortifacient efficacy of $\text{PGF}_{2\alpha}$, it did not improve the efficacy of hypertonic saline augmented with intravenous oxytocin. Patients aborted with $\text{PGF}_{2\alpha}$ and who received paracervical blocks aborted sooner (98% vs 70% at 32 hours) and with lower rates of incomplete abortion and gastrointestinal side effects compared to patients who did not receive paracervical blocks.

The mechanism by which the paracervical blocks affect the abortifacient efficacy of $\text{PGF}_{2\alpha}$ is not understood. The paracervical block probably does not interact with $\text{PGF}_{2\alpha}$ directly or affect the abortifacient efficacy of $\text{PGF}_{2\alpha}$ *per se* but probably brings about changes in cervical compliance. Extend-

ing the duration of the efficacy of the block (prolonging) probably enhances its effect on cervical compliance. The paracervical blocks may indirectly enhance the abortion process by alleviating pain associated with the procedure. With the alleviation of pain there could be a decrease in the output of epinephrine. Since epinephrine decreases uterine contractility which in turn may delay the abortion, the attenuation of epinephrine output may result in higher uterine contractility and reduced interval to abortion times. Twenty four hours after $\text{PGF}_{2\alpha}$ administration 12% of the patients who received paracervical blocks compared to 38.8% who did not had cervical dilation or effacement indicating that the paracervical block anesthesia may enhance $\text{PGF}_{2\alpha}$ in causing cervical softening, effacement and/or dilation. Hasson (8) has reported that topical application of lidocaine to the uterus cervical dilation may be easier and that the lidocaine may affect the internal cervical os.

For patients aborted with saline the cumulative abortion rates, rates of incomplete abortion, side effects and complications were similar regardless of whether or not paracervical block anesthesia was used. The cumulative abortion rates for patients aborted with $\text{PGF}_{2\alpha}$ and paracervical block anesthesia and for patients aborted with saline augmented with intravenous oxytocin were similar. The principal disadvantage of saline augmented with intravenous oxytocin is an increased incidence of cervicovaginal fistula (7), consumptive coagulopathy (2) and the possibility of water intoxication (6). For these reasons the use of effemim $\text{PGF}_{2\alpha}$ dose schedules may be preferable to that of saline augmented with high doses of intravenous oxytocin.

Further studies will be necessary to evaluate the effectiveness, safety and advantages of using longacting paracervical block anesthesia as an adjunct to midtrimester abortion procedures. These studies should include the evaluation of $\text{PGF}_{2\alpha}$ and its methyl analogues administered via the vaginal, intra amniotic, extra amniotic and intra muscular routes.

ACKNOWLEDGEMENT

The authors thank Dr Nelson Shub for assisting in the study and for his assistance in its design.

The study was supported in part by a grant from the Upjohn Company.

REFERENCES

- Brenner W E The current status of prostaglandins as abortifacients *Am J Obstet Gynecol* 123 306 1975
- Cohen E & Ballard C A Consumptive coagulopathy associated with intra amniotic saline instillation and the effect of intravenous oxytocin *Obstet Gynecol* 43 300 1974
- Comparison of intra amniotic prostaglandin F_2 and hypertonic saline for induction of second trimester abortion *Br Med J* 1 1373 1976
- Corlett R C & Ballard C A The induction of midtrimester abortion with intra amniotic prostaglandin F_2 *Am J Obstet Gynecol* 118 353 1974
- Edelman H A Brenner W E Mehta A C Philips F S Bhatt R V & Bhrawandiwala P A comparative study of intra amniotic saline and two prostaglandin F_2 dose schedules for midtrimester abortion *Am J Obstet Gynecol* 125 188 1976
- Goodlin R C McLennan C E Choyce J M Lee R S & Stickler J E Therapeutic abortion with hypertonic intra amniotic saline *Obstet Gynecol* 34 1 1969
- 7 Goodlin R Newell J O'Hare J & Steerz H Cervical fistula—a complication of midtrimester abortion *Obstet Gynecol* 40 87 1972
- 8 Grimes D A Schultz K F Cates W Jr & Tyler C W Jr Midtrimester abortion by intra amniotic prostaglandin F_2 Safer than saline? *Obstet Gynecol* 49 617 1977
- 9 Hasson H M Topical uterine anesthesia a preliminary report *Int J Gynaecol Obstet* (in press)
- 10 Motashaw N Personal communication

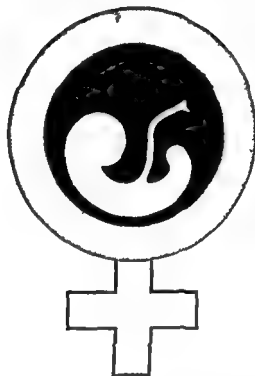
Submitted for publication August 16 1977

Leonard Laufé
International Fertility Research Program
Research Triangle Park
North Carolina 27709
USA

FOSTERÖVERVAKNING

Anencefali och Spina bifida
kan upptäckas redan på fosterstadiet

Serumanalys av alfafetoprotein (AFP)
i 16—19 graviditetsveckorna—
ett vardefullt hjälpmedel



Phadebas AFP PRIST[®]

(for kvantitativ bestämning av AFP i serum och amnionvatska)

Svensk dokumentation *

- teknik
- klinik

*B Kjessler & S G O Johansson Alpha fetoprotein (AFP) in early pregnancy
Acta Obstet Gynecol Scand Suppl 69(1977)

För information kontakta

Pharmacia Norden AB Avd Diagnostika Box 159 751 04 UPPSALA 018/11 11 00



Pharmacia

COMPARISON OF EXTRA AMNIOTIC INSTILLATION OF RIVANOL AND PGF₂ EITHER SEPARATELY OR IN COMBINATION FOLLOWED BY OXYTOCIN FOR SECOND TRIMESTER ABORTION

Anders Ölund and Bertil Larsson

From the Department of Obstetrics and Gynecology Karolinska Institutet Huddinge University Hospital Huddinge Sweden

Second trimester abortion was induced in 99 by extra amniotic instillation of Rivanol and/or followed by intravenous oxytocin after 24 hours instillations were made via a catheter with a balloon with 80 ml and left in place until abortion but never more than 24 hours. Induction was started by Rivanol (n=23) PGF₂ alone (n=23) Rivanol combined with PGF₂ (n=23) or Rivanol combined with half dose PGF₂ (n=23) and the patients were allotted to the different groups in a random manner. The Rivanol solution was used as a single dose but PGF₂ was instilled every 2nd or 4 hours. The mean induction-abortion time was 10 h in all 4 groups but a number of patients given Rivanol alone or in combination with Rivanol aborted earlier than patients induced by Rivanol alone during the 24 h before intravenous oxytocin was administered. Side effects were equally common after Rivanol as after PGF₂. With the methods and doses used in this investigation PGF₂ alone or combined with Rivanol and subsequent oxytocin had no overall advantage over Rivanol.

of serious complications. In most investigations oxytocin has been used as supplementary therapy (3-5-10) and Rivanol has been used alone in only a few reports (6).

The use of prostaglandins as abortifacients was first reported in 1970 (7-14). The advantages of the method were a shorter induction-abortion interval and the absence of fatal complications. A serious disadvantage, however, was the high incidence of gastrointestinal side effects, especially when the substance was given by the intravenous route (8). This incidence has, however, been reduced by the use of the intrauterine route and new prostaglandin analogues (8).

The purpose of the present study is to evaluate the usefulness of Rivanol and PGF₂ administered extra amniotically either alone or combined and with subsequent oxytocin for induction of abortion with special reference to the induction-abortion interval and side effects.

PATIENTS AND METHODS

The material consists of 92 apparently healthy women admitted to hospital for legal abortion on social reasons. The patients were randomly distributed into four treatment groups, each consisting of 23 patients. Table 1 gives the distribution of the age, parity and number of previous legal abortions as well as the gestational age estimated from uterine size.

Induction was started by the introduction of a Foley catheter No. 20 via the cervical canal into the extra-ovular space just inside the internal cervical orifice. The Foley balloon was filled with 30 ml of physiological saline. Rivanol and/or PGF₂ was then instilled (see below). The catheter was left in place for 24 hours or expelled with the fetus if the patient aborted earlier. If the abortion had not started by the following morning, an intravenous infusion of oxytocin 70 IU in 1000 ml of 5% glucose was started. This infusion was given at the rate of 1000 ml every 12 hours until abortion occurred. The patients were

or extra amniotic instillation of hypertonic saline solutions for induction of second trimester abortion has been the method most commonly used in Scandinavia for the last two decades. Although the method is generally effective it may lead to even fatal complications (1-15). In Japan, because of reports of maternal deaths from the use of hypertonic saline solutions (15) this has been replaced by extra amniotic instillation of an 0.1% solution of Rivanol (6,9) or 16-methyl-19-nor-pregn-20-ene-20-carboxylic acid lactate. It was found to be equally effective as hypertonic saline and has been used in Japan for more than 20 years without any known serious side effects (11). Rivanol has been used successfully in Sweden since 1969 (6). In a comparison of the efficacy of the hypertonic saline and Rivanol methods, respectively, Ingemansson (6) reported a shorter induction-abortion interval for Rivanol as well as the absence

Table I Distribution of age, parity, earlier legal abortion and gestational age

Group	Age		0-para	X para	Earlier legal abortion	Week of gestation clinical examination		
	Mean	Range				Mean	Range	S
1 Rivanol ($n=23$)	24	14-40	7	16	6	15.1	13-19	1
2 PGF ₂ ($n=23$)	24	16-34	9	14	7	16.2	13-4	2
3 Rivanol+PGF ₂ ($n=23$)	25	16-41	8	15	7	17.0	13-25	3
4 Rivanol+ $\frac{1}{2}$ PGF ₂ ($n=23$)	24	15-44	10	13	5	16.4	13-24	1

closely observed throughout the procedure and side effects if any were carefully recorded.

The following substance were instilled in the extra-ovular space via the catheter:

Group 1 Rivanol

A 0.1% solution of Rivanol was slowly instilled 10 ml per gestational week but never more than 150 ml after which the catheter was ligated at its lower end.

Group 2 PGF₂

1 ml of a saline solution containing 0.25 mg PGF₂ per ml was instilled followed by 3 ml every 2nd hour for up to 24 hours. At a gestational age of more than 16 weeks the dose was doubled and given at the same intervals.

Group 3 Rivanol + PGF₂

Rivanol was instilled as in group 1 followed by PGF₂ as in group 2.

Group 4 Rivanol + $\frac{1}{2}$ PGF₂

Rivanol and PGF₂ was instilled as in group 3 except for the dose of PGF₂ which was reduced by half.

RESULTS

There was no significant difference in the clinical characteristics of the groups (Table I) as regards age, parity, number of earlier abortions and weeks of gestation (evaluated by mean of Student's *t* test and the chi square test).

The cumulative abortion rates of the four groups are graphically illustrated in Fig. 1. Patients given PGF₂ (groups 2, 3 and 4) in a number of cases aborted earlier than patients treated with Rivanol alone (group 1). The cumulative abortion rates from 30 hours after induction were essentially similar in all groups.

The mean abortion times were in group 1 29.9 hours (range 23.9-47.2) in group 2 26.7 hours (range 8.9-63.0) in group 3 24.9 hours (range 9.3-66.1) and in group 4 26.5 hours (range 13.1-50.1). The differences in mean abortion time

between any two of the four groups were statistically significant (Student's *t* test).

Table II gives the complications and side effects. The small number of patients with endometrial excessive blood loss not large enough to warrant statistical comparison seems to be equally distributed among the groups. Gastrointestinal side effects were common in all four groups. The somewhat higher incidences in group 2 and 3 did differ significantly (chi square test) from the other two groups.

DISCUSSION

The induction-abortion interval in the Rivanol group (group 1) of the present study (83% within 36 hours, 100% within 48 hours) is of the same order of magnitude as that given in previous reports. Thus Ingemansson (6) reported 77% within 48 hours and 94% within 72 hours and Himmelmann et al. (5) 77% within 48 hours and 100% within 72 hours.

The shorter abortion time observed in the present study might be explained by the difference in methods used. Ingemansson (6) used a catheter with a balloon and introduced it almost completely into the uterine cavity after the instillation of Rivanol but did not use subsequent intravenous ytocin. Himmelmann et al. (5) used a Foley catheter.

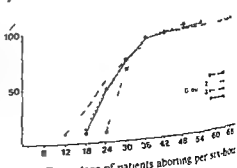


Fig. 1 Percentage of patients aborting per six-hour interval of time.

c II Complications and side effects

	Groups			
	1 Rivanol (n=23)	2 PGF ₂ (n=23)	3 Rivanol+ PGF ₂ (n=23)	4 Rivanol+ xPGF ₂ (n=23)
metritis	2 (8.7%)	1 (4.4%)	3 (13%)	3 (13%)
loss with transfusion	1 (4.4%)	2 (8.7%)	2 (8.7%)	3 (13%)
intestinal side effects				
nausea	17 (57%)	26 (70%)	16 (70%)	13 (57%)
vomiting	7 (30%)	13 (57%)	13 (57%)	11 (35%)
diarrhoea	10 (44%)	11 (48%)	9 (39%)	11 (52%)
anuria	0	2 (8.7%)	0	1 (4.4%)

pped with a balloon filled with only 10 ml one l of the volume used in our study and administered oxytocin at 24 and if necessary at 48 s. hat the presence of a balloon catheter reduces induction-abortion interval has been demonstrated by Fylling & Refsdal (3). Moreover Manabe induced abortion with only a metreurynter d with 150 ml saline solution followed by intravascular injection of oxytocin. With the aid of a hod similar to that used by Manabe except for use of a balloon catheter filled with only 40 ml responding results have been obtained by Ölund & Brandel (to be published). These observations emphasize the importance of the balloon per h the induction of abortion.

he underlying mechanism of the abortifacient et of Rivanol is still obscure. An oxytocic effect elated acridin dyes has however been reported. Furthermore in abortions induced with hy onic saline a stimulation of the release of endogenous PGF₂ from the decidua has been produced by Gustavii (4). A similar release of PGF₂ in duon induced by Rivanol was suggested by n et al (12). In the present investigation the initial lag time and the high incidence of intestinal side effects after induction with ol lend support to such a possibility.

material the method including only PGF₂ duced abortion in 48% within 24 hours and in r within 36 hours (Fig. 1) figures resembling achieved with comparable methods. Embrey al (7) for instance adding oxytocin after 36 noticed 67% abortions within 24 hours and within 36 hours.

to the present study PGF₂ alone or combined Rivanol induced abortion within 24 hours more than Rivanol alone (Fig. 1). Regarding the

abortion rate after 24 hours when oxytocin was added the results of induction with PGF₂ combined or alone showed no benefits over the abortion rate with Rivanol. Furthermore the PGF₂ methods involves repeated instillation of the drug a risk of cervico vaginal injuries (13) and bronchoconstriction (16).

Even if there is a initial lag time of approximately 24 hour in the Rivanol method the mean abortion time is not significantly longer than in the PGF₂ methods.

Rivanol has also few if any contraindications and there has been no reports of cervico-vaginal injury with the doses used in the present study.

Judging from the observations made in the present series the use of the Rivanol method as applied here means a safe as well as a simple way for induction of midtrimester abortion. It would appear that PGF₂ alone or combined with Rivanol with subsequent oxytocin after 24 hours as used in the present study had no definite over all advantage over the Rivanol method.

REFERENCES

- Berger S, Tietze C, Pakter J & Katz S H. Maternal mortality associated with legal abortion in New York State. *Obstet Gynecol* 43: 315-36, 1974.
- Embrey M H, Hilker K & Manhendran P. Induction of abortion by extra amniotic administration of prostaglandins E₂ and F₂. *Br Med J* 13: 146-149, 1972.
- Fylling P & Refsdal A. Rivanol induced mid trimester abortion. *Arch Gynaecol* 215: 363, 1973.
- Gustavii H. Sweeping of the fetal membranes by a physiologic saline solution: effect on decidual cells. *Am J Obstet Gynecol* 170: 531-536, 1974.
- Hummelmann A, Myhrman P & Svanberg S H. Induction of second trimester abortion. Comparison between Rivanol and prostaglandin F₂ regarding time factors and complications. *Contraception* 12: 645-654, 1975.

- 6 Ingemansson C A Legal abortion by extra amniotic instillation of Rivanol in combination with rubber catheter insertion into the uterus after the twelfth week of pregnancy *Am J Obstet Gynecol* 115 211-215 1973
- 7 Karim S M M & Filshie E Therapeutic abortion using prostaglandin F_2 *Lancet* i 157-159 1970
- 8 Karim S M M & Amy J J In *Prostaglandins and Reproduction* (ed S M M Karim) p 77-148 MTP Press Ltd Lancaster 1975
- 9 Lewis B V Pybus A & Stillwell J H The oxytocic effect of acridine dyes and their use in terminating mid trimester pregnancy *J Obstet Gynaecol Br Commonw* 78 838-842 1971
- 10 Manabe Y Metreurylter induced abortion at midpregnancy *Am J Obstet Gynecol* 99 557-561 1967
- 11 Manabe Y Artificial abortion at midpregnancy by mechanical stimulation of the uterus *Am J Obstet Gynecol* 105 132-146 1969
- 12 Martin J M Bygdeman M Leader A & Wijkvist N Early second trimester abortion by the extra amniotic instillation of Rivanol solution and a single PGF_2 dose *Contraception* 11 523-531 1975
- 13 Purandare V N Ganguli A C Chatterjee R M & Krishna U R Cervico-vaginal injuries in cases of second trimester termination of pregnancy Prostaglandins 13 349-354 1977
- 14 Roth Brandel V Bygdeman M Wijkvist N & Bergström S Prostaglandins for induction of therapeutic abortion *Lancet* i 190-191 1970
- 15 Wagatsuma T Intra amniotic injection of saline for therapeutic abortion *Am J Obstet Gynecol* 117 743-745 1965
- 16 Weir E K Greer B E Smith S C Silver G W Droegemueller W Reeves J T & Grover L F Bronchoconstriction and pulmonary hypertension during abortion induced by 15 methyl prostaglandin F_2 *Am J Med* 60 556-560 1976

Submitted for publication May 24 1977

Anders Olund
Department of Obstetrics and Gynecology
Huddinge University Hospital
S 141 86 Huddinge
Sweden

ULTRASTRUCTURE AND VARIATIONS OF HUMAN CERVICAL MUCUS DURING PREGNANCY AND THE MENOPAUSE

F C Chretien

Laboratoire d'Histologie-Embryologie-Cytogenetique Hopital de Bicetre France

Ultrastructural aspect and variations of cervical mucus have been studied by scanning electron microscopy in 58 pregnant and 29 menopausal women. In both groups a striking tightening of the ultrastructural protein framework was demonstrated. The phenomenon appears to occur very rapidly at the beginning of pregnancy while patients near the menopause show more variable patterns. The results are compared to the known evolution of cervical mucus ultrastructure during the ovarian cycle and discussed from a rheological point of view. Particular attention was paid to the eventual bacterial role of cervical mucus in pregnancy which is found to be very plausible.

During pregnancy the uterine cervix undergoes important physiologic changes under hormonal influence. The most striking modification occurs in the endocervical mucosa and glands. At the epithelial level a cellular proliferation is manifested by numerous areas of active cell multiplication and by stratification of the tall columnar cells (12). The glands grow larger, become gradually more numerous and more active as pregnancy proceeds. Occasionally hyper trophy and hyperplasia are extreme. The glandular stroma becomes oedematous and vascularized and it would appear that there is lessened support for the glands.

Even though rather small amounts of cervical mucus can be sampled during that period, the mucus secretion is noticeable from the beginning to the end of pregnancy (13). In addition, no significant variation can be observed in secretion rate dependent on the number of children or parity of pregnancy (3). However, during pregnancy the cervical mucus undergoes drastic variations modifying both its physico-chemical properties and its physical appearance.

From a macroscopic point of view, cervical mucus during pregnancy is known to become increasingly thick, sticky and gelatinous, therefore simi-

lar to that seen premenstrually during the time of normal luteal activity (2). Thus appears at the external os a clot of dense mucoid material which obstructs the cervical canal. The physiological significance of this mucus thickening, which causes an important decrease of its spinability, has not yet been established. However, bacterial invasion of the uterine cavity during pregnancy is known to be extremely rare. Beck (1) stated that thick cervical mucus in the canal during pregnancy aids in preventing the ascent of pathogenic bacteria into the uterine cavity. The fact that the tightening of cervical mucus framework could create a physical barrier to the largest bacteria is now made more plausible by the most recent information on cervical mucus ultrastructure. Due to its depth of field, the scanning electron microscope can provide a three-dimensional view of the spatial arrangement of the infrastructure of cervical mucus and shows it as a meshwork of intertwined filaments (8) stretching or thickening during the ovarian cycle depending on hormonal influence (7). Indeed, progesterone is known to cause the mucus to thicken whereas oestradiol has the opposite effect. The results given in a preliminary report have shown that cervical mucus during pregnancy and after the menopause can have an ultrastructural appearance similar to that described for the late luteal period (6). The important thickening of cervical mucus could be due to the shrinkage of its infrastructure under the effects of progesterone domination during the same periods.

The following study with the scanning electron microscope was carried out in order to provide further information on the variations of cervical mucus framework throughout pregnancy and during the menopause. Particular attention was paid to the possible mechanical antimicrobial role of cervical mucus during pregnancy.

Table I

Gestation duration	Number of cases	
	Primi gravidas 27	Mult gravidas 31
3 weeks to 1 month	3	4
5 weeks	4	3
2 to 3 months	5	4
5 to 6 months	3	4
7 to 8 months	4	5
8½ months	4	5
Last week of pregnancy	4	6

METHODOLOGY

In both pregnant and menopausal patients mucus samples were secured from cervixes that were apparently healthy and did not reveal obvious erosions. The cervix was exposed with a speculum in a good light and the ectocervix gently wiped with sterile gauze to carry away vaginal debris and old mucus. The mucus was taken by aspiration by means of a plastic Braun probe adapted to a 10 ml disposable syringe. After the external opening had been cleaned with cotton the probe was introduced into the cervical canal. The mucus was then aspirated very carefully in order to avoid the formation of bubbles which could create alterations in the native structure of the sample. Only those specimens which had not been submitted to strong suction or ejection were set aside for observation. At least two samples per donor were taken.

The mucus was placed on glass coverslips, then prepared for scanning electron microscopy including freeze drying according to the procedure described previously in a detailed technical paper (5). The samples were coated with either pure gold or an alloy of 80/20⁹⁹ gold/palladium.

Scanning observations were conducted with Cambridge Stereoscan Mk II and Etec Autoscan Microscope under an accelerating voltage of 25 and 20 kV respectively.

RESULTS

Pregnancy

The mucus specimens used for this study were taken from 58 pregnant patients between 19 and 39 years of age distributed between primigravidas and multigravidas with a gestation range from 3 to 38 weeks (Table I).

The observation of 153 mucus samples showed that the filamentous wool constituting its solid phase persists throughout the pregnancy. Indeed during this period the observed ultrastructural organization of cervical mucus appears to be not fundamentally different from certain aspects de-

scribed in the mucus sampled during the cycle out of the ovulatory period (7).

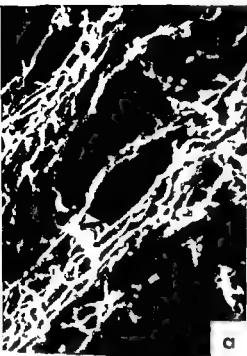
The same types of filaments can framework with the exception of the α type filaments (α type) which are absent to the end of pregnancy. In addition revealed an obvious evolution in exceeds of both the length and aspect of the filaments as well as in the dimension of the they constitute.

In order to present the results in pregnancy was divided into 6 periods: 3 weeks 2½ months 5-6 months 7-8 week of pregnancy. This sequence was from the fact that the differences are cant from one month to the following in part of pregnancy. Therefore there was analyse each of the nine months in characteristic aspects of each of the will be described in succession.

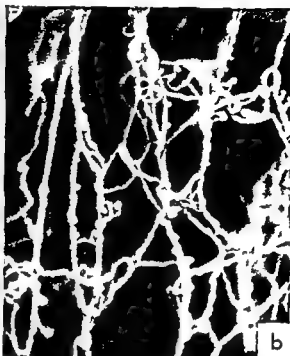
3 weeks At the beginning of pregnancy mucus framework appears grossly similar to seen at the beginning of the luteal phase. Filaments are largely predominant and γ only occasional. The wool frequently and shows filaments which can be smooth of their length but also show median and expansions. They can form meshes of 1 sion (up to 7 μ m) and may appear 1b). The mucus frequently shows in heterogeneity of the wool density areas it is possible to find some sports framework more dense forms meshes of dimensions (Plate 1a).

5 weeks Two weeks later the general the framework is not greatly different in heterogeneity can be demonstrated in the the largest meshes may attain 6 μ m (Plate However as compared with the preceding the median thicknesses puffing up certain γ appear larger and areas of obvious compact be seen here and there in the sample (Plate 1d).

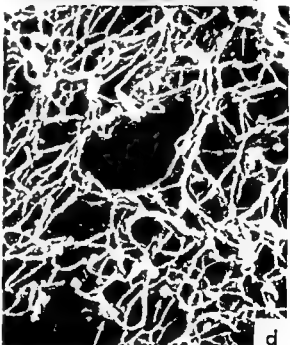
2½ months This time is an important stage evolution of the cervical mucus framework during pregnancy. Indeed if meshes of appreciable size can be observed in the thin marginal area of samples the most part of the wool appears now dense and homogenous (Plate 2a). Most of the β filaments have considerably shortened and appear to be roughly rectilinear. The γ filaments are still occasional and only visible in few places. Lateral



a



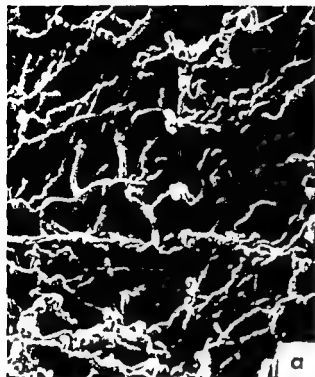
b



d

(a) 19th day of pregnancy. The wool in which
 meshes can be seen appears heterogenous $\times 10800$
 day of pregnancy detail showing large meshes
 c) 5 weeks of pregnancy. At low magnifica-

tion the wool heterogeneity appears particularly evident
 $\times 4800$ (d) 5 weeks of pregnancy. Detail showing straight
 and buckled filaments some of which appear puffed up
 by median thicknesses (arrows) $\times 10700$



a



b



c

Plate 2 (a) 2½ months of pregnancy The wool appears considerably tightened $\times 10\,000$ (b) 2½ months of pregnancy Detail showing the deformed filaments and a characteristic clot (arrow) $\times 20\,000$ (c) 4 months of preg-

nancy Low magnification showing the homogeneity of the wool at that stage $\times 4\,000$ (d) 5 months of pregnancy Detail $\times 11\,500$

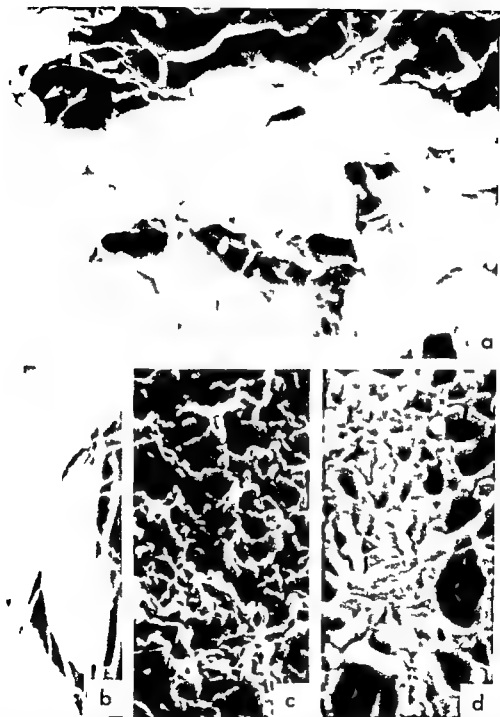


FIGURE 3. (a) 8 months of pregnancy. Detail of the structural wool in which several clots are visible (arrows) $\times 1700$. (b) Last weeks of the pregnancy. In this particular case cervical mucus presented in certain areas a preovulatory aspect. Note the long and smooth filaments $\times 13000$. (c) Last week of the pregnancy. In most cases the mucus framework appears to be extremely dense $\times 10800$. (d) Last week of the pregnancy. Detail. Most meshes can scarcely be distinguished $\times 1400$.

Table II

Pregnancy	3 weeks	5 weeks	24-4 months	5-7 months	8 months	Last week
Density of the wool (+ to +++++)	+-	++	++++	++++	+++	++++
Appearance of filaments						
Smooth	++	++	0	0	0	0
Buckled	++	++	0	0	0	0
Straight	+	+	++	++	++	++
Puffed up	+	+	++	++	++	++
With clots	0	0	+	+	+	++
Types of filaments						
α	0	0	0	0	0	0
β	+++	++++	++++	++++	++++	++++
γ	++	++	+	+	+	+

median expansions are frequent along the β filaments which can also be expanded by clots (Plate 2b). The mesh dimensions which are difficult to measure rarely exceed $1\ \mu\text{m}$. No really significant variation can be observed until 5 months.

5-6 months During this period the mucus framework is particularly dense and compact (Plate 2c). The filaments appear very short and puffed up by successive median or lateral expansions (Plate 2d). The γ filaments extremely rare can be seen here and there through some of the meshes formed by the β filaments. The dimension of the meshes which are very homogenous rarely goes beyond $1.3\ \mu\text{m}$. Until the end of the seventh month the meshwork shows only slight variations in amplitude thought to be of little significance. Thus the general aspect of the wool between 5 and 6 months of pregnancy is not fundamentally different and it is difficult to even distinguish between these different stages.

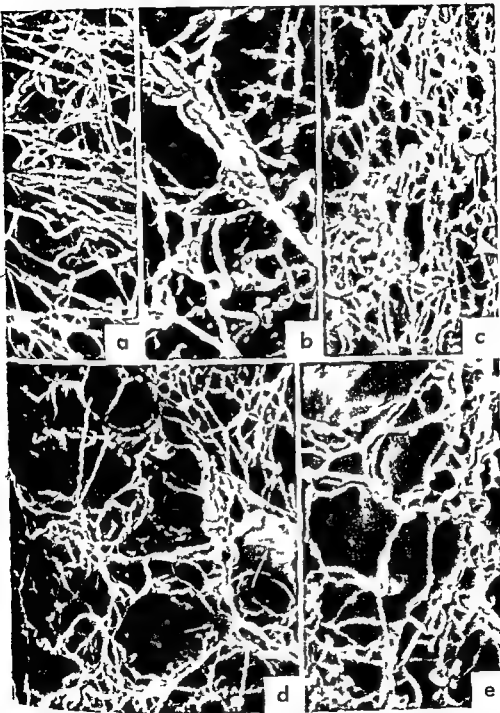
7-8 months In approximately 50% of samples the framework of cervical mucus sampled in the 8th month of pregnancy seems to be a little less homogenous than previously. The wool is still compact but in some places the β filaments constituting the main part of the meshwork can appear to be slightly slackened. The median and lateral expansions frequent in the dense parts of the wool are here rather rare giving the filaments an almost smooth appearance (Plate 3a).

Last week of pregnancy The framework of

cervical mucus sampled during the last pregnancy is generally conspicuous for its compactness (Plate 3b). Indeed the mesh appears so closely tightened that they give which looks fairly uniform the aspect of rough concrete (Plate 3c, d). Due to this density of the wool most of the meshes exceed $0.3\ \mu\text{m}$. The filaments are puffed and deformed by expansions and that they may blend and constitute clots at the surface of the wool. However in the mucus framework was shown to be in some places distributed at the periphery of the sample the filaments appeared elongated (Plate 3b). In these cases the framework was similar in appearance to the preovulatory period. Nevertheless that kinkage seems to be very rare and h

Table III

Pre-menopause	1½ to 3 months between the two last menses
Menopause	4 to 6 months between the two last menses
Post-menopause I	7 months to 4 years since the last menses
Post-menopause II	More than 4 years since the last menses



to 4 (a) Premenopause (48 years old) Mucus sampled the 1st day of a 36-day cycle. The framework has a reticular appearance $\times 6300$ (b) Premenopause (45 years old) Mucus sampled on the 26th day of a 30-day cycle. Note that the filaments are arranged in a luteal type mesh $\times 6300$ (c) Premenopause (47 years old) Mucus sampled on the 1st day of a 45 day cycle. The meshwork

appears here to be very dense. Some clots are protruding at the surface (arrows) $\times 5000$ (d) Menopause (49 years old) 4 months since the last menses period. Some clots are clearly visible (arrows) $\times 10700$ (e) Menopause (same sample) Detail of particularly large meshes $\times 11400$

Table IV

	Pre menopause	Menopause	Post menopause I	Post Menopause II
Density of the wool (+ to +++++)	Variable from + to +++++ according to stage of the cycle	+++	++++	+++++
Appearance of the filaments				
Smooth		0	0	0
Buckled		0	0	0
Straight	idem	++	++	++
Puffed up		++	++	++
With clots		+	++	++
Types of the filaments				
α	+	0	0	0
β	+++++	+++++	+++++	+++++
γ	++	+	+	+

considered occasional. The most striking differences occurring into cervical mucus framework during pregnancy are summed up in Table II.

Menopause

The mucus specimens were sampled from 29 women between the age of 45 and 69 years coming to the hospital for routine examination. The patients were divided into 4 groups according to the stage of menopause (Table III). A total of 64 samples was observed.

As was shown during pregnancy after the onset of the menopause the infrastructural wool of cervical mucus appears to be comparable to that described for the early luteal phase. Then the meshwork tightens very rapidly to form a more and more compact structure.

Pre menopause Generally the ultrastructural appearance of cervical mucus sampled at the beginning of the menopause appears to depend on the stage of the cycle at the time of sampling. Indeed near midcycle filament arrangements resemble those of normal ovulatory type (Plate 4a). Whereas samples taken at the beginning or the end of cycle exhibit a tight meshwork similar to that of normal follicular or luteal phase (Plate 4b, c).

However in most samples the wool density can exhibit a certain heterogeneity which does not seem to be related to the day of sampling. For instance in

a luteal type mucus one can find some areas where the filaments appear smooth and even buckled. The dimension of the meshes is thus very diversified and may vary between 8 and 0.3 μ m. α filaments are absent and γ filaments only occasional.

Menopause The framework is now more and rather homogenous. It is composed of short and thicker β filaments. Most of these filaments appear to be smooth and roughly rectilinear (Plate 4d, e) but they can form some clots protruding from the surface of the wool (Plate 4d). The mesh dimension rarely exceeds 3 μ m.

Post menopause I The most part of the meshwork appears to be particularly tight (Plate 4a). The wool is now composed of very short β filaments which are frequently distorted by median nodes and clots originating from lateral buds (Plate 4b). The size of the meshes may vary between 1.5 and 0.3 μ m.

Post menopause II The wool is now uniform so dense that most meshes can scarcely be distinguished. The filaments exhibit numerous median swellings and lateral buds. Large clots are not rare. The mesh may attain 0.8 μ m but the main dimension does not exceed 0.3 μ m. To make the results more clear the most characteristic data concerning the evolution of the cervical mucus framework during the menopause are summed up in Table IV.

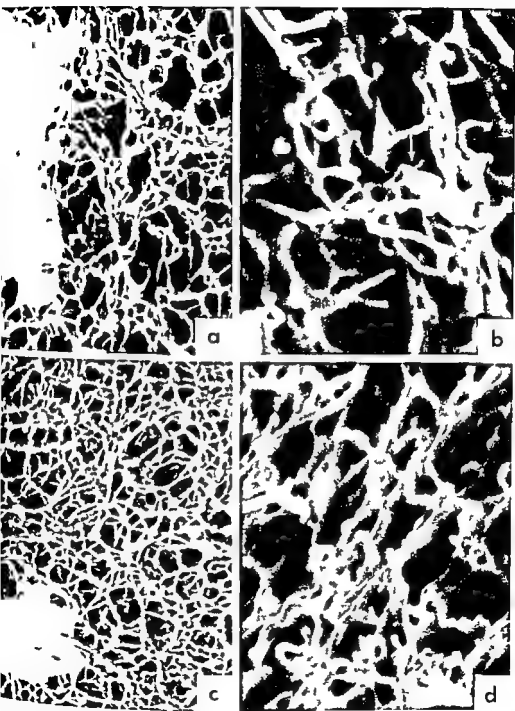


Fig. 3. (a) Over menopause I (53 years) One year since the last menses period. The wool appears to be considerably tightened compared with the preceding stage. (b) Over menopause I (51 years) 3 years since the last menses period. Detail showing the compactness of the meshwork. The arrow indicates a typical clot. (c)

Over menopause II (69 years) 19 years since the last menses period. The wool is extremely dense. (d) Over menopause II (66 years) 15 years since the last menses period. Note the extreme compactness of the meshwork.

DISCUSSION

The importance of cervical mucus in human female reproductive physiology makes it essential to study its macromolecular organization and identify the mechanisms that modify this organization during the various parts of sexual life. The scanning electron microscope seems incontestably to be now the best available instrument for the study of the three dimensional infrastructure of mucoid secretions, but the interpretation of the pictures obtained remains particularly delicate. Indeed preparation of cervical mucus for observation within a vacuum chamber must surmount several technical barriers. Probably the most important is to eliminate free water contained in the specimen so that it will not vaporize under the high vacuum of the specimen chamber of the microscope. In fact eliminating the important amount of water or cervical mucus (up to 95%) without inducing major changes in the native structure of such a plastic material can be considered as a challenge.

Since the SEM studies of cervical mucus are very recent it is logical to ask if the data they provide are reliable. The stage of fixation and dehydration are notably prone to produce many possible artifacts and one may ask whether the micrograph patterns produced on cervical mucus are really natural structures. I refer the interested readers to a detailed paper devoted to the technical problems involved in cervical mucus preparation and observation under SEM (5).

Due to its great plasticity, cervical mucus ultrastructure may undergo local rearrangement as a result of local accidental physical constraints. Thus parameters such as mesh dimension, length, mean number per unit of surface of a certain type of filament cannot be measured accurately. Therefore in Tables II and IV symbols such as + or - have been used instead of numbers for permitting quantitative evaluation.

Although the infrastructural framework is probably altered by preparative procedures there is in fact much evidence that the pictures obtained can be considered the most faithful illustration of the natural arrangement of the structural wool in cervical mucus.

Several arguments can be proposed in support of that view. At first the structures described previously for the human ovarian cycle resembled none of the characteristic artifacts of which we are aware

in scanning electron microscopy. The parallel between their variations in the course of the cycle and the theoretical variations proposed by Oates (18) or the basis of nuclear magnetic resonance studies (17) appears too close to be simple coincidence.

Moreover being able to produce at will on the follicular and luteal type pictures in the cervix of castrated baboons by injecting oestrogen and progesterone (9) clearly shows that the observed structures lie in hormonal stimulation and cannot result from technical manipulation. Finally the results obtained with mucus sampled from women during pregnancy and during or after the menopause corroborate the data of macroscopic observation and also correspond to the modification of mucus framework one can logically expect from the hormonal balance during these periods.

Following conception the obstruction of the cervical canal by a clot of very thick mucus appears to be a very rapid phenomenon. The abrupt changes leading to the formation of an extremely dense mucus framework are consistent with the notion that cervical mucus during pregnancy acts as an effective filter. In less than 10 weeks the structural wool becomes similar to that of the late luteal phase thus creating a physical barrier to sperm cells (19). The density of the wool then quickly reaches a maximum with only slight variations in amplitude thought to be of little significance.

The question arises now whether cervical mucus during pregnancy is able to protect the uterus against invasion from microbial invasion as evidenced from the small number of infections of the upper genital tract. It indicates that besides the reticulo-humoral defence mechanism there may exist a physical obstacle created in the uterine cervix which prevents bacterial and fungal invasion from the vagina. It is reasonable to envisage a cervical mechanical barrier. On the basis of my observations I can assume that such a mechanism is highly probable. It is well known that during pregnancy the penetration of cervical mucus by sperm cells is restricted. The spermatozoa are mobilized (19). Of course due to the size of the meshes only largest bacteria or fungi could be stopped mechanically by the wall. However the superimposition of many layers of meshes presents to large microbial agents a series of obstacles through which they finally become trapped.

Thus after some weeks of pregnancy the cervix

could act as an effective filter making impossible the ascent of bacteria and fungi towards the uterine cavity. Probably the antimicrobial action of cervical mucus during pregnancy does not take place only by the rapid tightening of the meshwork. Other mechanisms are likely to interfere. During pregnancy, cervical mucus has been shown to contain histiocytes, plasmacytes, eosinophils and nucleate cells, which probably contribute to the bacterial protection of the uterine cavity (3). The particular arrangement of altered histiocytes, polymorphonuclear cells on the periphery of stretched cervical mucus and the fact that the central zone contains many normal and healthy cells is probably related with the antimicrobial defense of the uterine cavity during pregnancy.

Moreover, the presence of muramidase (or lysozyme) in cervical mucus of pregnant woman is likely also to contribute to that defence. Indeed, this enzyme, probably secreted by the endocervix, has been shown able to hydrolyze the wall of both Gram positive and negative bacteria (11) and thus to facilitate their phagocytosis inside cervical mucus (3, 14).

During the first third of pregnancy, in certain portions of the same sample, the web appears more heterogeneous than in the others. This heterogeneity probably corresponds at the ultrastructural level to that which one can sometimes observe visually when measuring the cervical mucus spinability with an automatic device (10). Indeed, cervical mucus samples at the beginning of pregnancy can exhibit one or several nodosities of different viscosity. Probably during this period, cervical mucus consists of a mixture of chemically different forms which are produced simultaneously by different cervical glands in the case during the ovulatory cycle (20).

The rapid tightening of the ultrastructural web in the beginning of pregnancy is not surprising. In fact, cervical mucus contains a smaller amount of water during pregnancy than during the luteal phase (16). Spinability is known to be dependent on water content.

Very likely, the tightening of the mucus framework can be accounted for by a particular biological property of cervical mucus during pregnancy: that property, plasticity, enables cervical mucus to be deformed continuously and permanently without rupture (4). It is characteristic enough to permit diagnosis of pregnancy in 96% of 155 mucus samples (21).

Cervical mucus of menopausal women before estrogen therapy was known to have scanty appearance, to be highly viscous or crumbly in character, cloudy white or yellow, and to have a heavy content of epithelial cells and leucocytes. Moreover, it has been shown to be impenetrable by spermatozoa *in vitro* (22). The considerable tightening of the mucus framework demonstrated by scanning electron microscopy can evidently account for that latter property. The preovulatory aspect presented by cervical mucus in some early menopausal patients must not be considered surprising. Oestrogenic analyzes made during the first few months after the menopause may show values similar to those obtained in women in child bearing age (2). In fact, senile retrogression of the ovaries is apparently first attended by cessation of corpus luteum function and later by decreased estrogenic secretion.

Thus, concerning both pregnancy and menopausal cervical mucus, scanning electron microscopic observations fully corroborate at the ultrastructural level the known macroscopical data. They also explain or demonstrate some of their supposed physiological properties. One can state now that scanning electron microscopy has proved itself a particularly useful instrument for reproduction research, whose performance complements that of the transmitting electron microscope. It also opens a new field of research for the study of mucoid materials whose essentially lacunar or fibrillary nature may make observation impossible with the classical electron microscope.

ACKNOWLEDGMENTS

Original research of this kind could never be done without the help and advice of senior clinicians. I wish to acknowledge the help of Professor P. Engelmann (Hôpital Lariboisière) and Doctor J. Cohen with the collection of cervical mucus samples. For his very valuable criticism, I am indebted to Professor G. David (Hôpital de Bicêtre). My thanks are also due to Doctor S. Segal for his kindness in making available the excellent facilities of the Population Council (Rockefeller University, New York) and to Professor M. Bessis for kindly permitting the use of the scanning electron microscope of the Institut de Pathologie Cellulaire-Inserm U48 (Hôpital de Bicêtre).

REFERENCES

1. Beck, A. C. *Obstetrical Practice*. William and Wilkins, Baltimore, 1942.

- 2 Bergman P Sexual cycle time of ovulation and time of optimal fertility in women. Studies on basal body temperature, endometrium and cervical mucus. *Acta Obstet Gynecol Scand* 29 (Suppl 4) 5 1950
- 3 Bret A J & Coiffard P Le mucus cervical au cours de la grossesse. In *Les fonctions du col uterin* p 225 Masson et Cie Paris 1964
- 4 Clift A F Observations on certain rheological properties of human cervical secretion. *Proc Roy Soc Med* 39 1 1945
- 5 Chretien F C Preparation du mucus cervical a l'observation au microscope électronique à balayage. *J Microscopie Biol Cell* 24 23 1975
- 6 Chretien F C Cohen J Borg V & Psychoyos A Human cervical mucus during the menstrual cycle and pregnancy in normal and pathological conditions. Proceedings of the 8th World Congress of Fertility and Sterility Buenos Aires 1974. *J Reprod Med* 14 192 1975
- 7 Chretien F C Cohen J & Psychoyos A L'évolution de la trame ultrastructurale de la glaire cervicale humaine au cours du cycle ovarien. Etude au microscope électronique à balayage. *J Gynecol Obstet Biol Repr* 5 313 1976
- 8 Chretien F C Gernigon C David G & Psychoyos A The ultrastructure of human cervical mucus under scanning electron microscopy. *Fertil Steril* 24 746 1973
- 9 Chretien F C Olmedo C & Psychoyos A Unpublished data 1977
- 10 Chretien F C Ozenda B & Volochine B An automatic device for measuring cervical mucus spinnability. *Med Biol Eng & Comput* 15 673 1977
- 11 Davis St D Gemsa D Janetta A & Wedgwood R J Potentiation of serum bactericidal activity by lysozyme. *J Immunol* 101 277 1968
- 12 Fluhman C F A clinical and histopathologic study of lesions of the cervix uteri during pregnancy. *Am J Obstet Gynecol* 55 133 1948
- 13 Gandolfo-Herrera R & Bears B B The cervical mucus in pregnancy. *Obstet Gynecol Lat Amer* 11 1953
- 14 Govers J & Girard J P Some immunological properties of human cervical and vaginal secretions. *Gynecol Invest* 3 184 1977
- 15 Guttmacher A F & Shettles L B Cytochrome in cervical mucus and its practical importance for human fertility. 5 4 1940
- 16 MacDonald E R Cyclic changes in cervical mucus. *J Obstet Gynecol Br Commonw* 76 1090 1969
- 17 Odeblad E Micro NMR in high permanent magnetic fields. Theoretical and experimental investigation with application to the secretions from a glandular unit in the uterine cervix. *Acta Obstet Gynecol Scand* 45 (Suppl 7) 177 1961
- 18 Odeblad E The functional structure of human cervical mucus. *Acta Obstet Gynecol Scand* 45 (Suppl 1) 59 1968
- 19 Palmer R La stérilité involontaire. Masson et Cie Paris 1950
- 20 Rudolfsson C Nuclear magnetic resonance and cytometric studies on mucus from single cervical glands. *Int J Fertil* 16 147 1971
- 21 Scott Blair G W Cowie A T & Copen F W Rheological properties of secretions from the cervix of pregnant and non pregnant cows. *Nature* 149 1947
- 22 Shettles L B & Guttmacher A F Normal and abnormal variations in human cervical mucus. *Physiol* 129 467 1940

Submitted for publication Febr 21 1977

F C Chretien
Histologie Embryologie-Cytogénétique
Centre Hospitalo universitaire de Brest
78 Avenue du Général Leclerc
94700 Le Kremlin Bicêtre
France

FRACTURE AND CHEMICAL COMPOSITION OF THE DEPOSIT FORMED ON THE LIPPES LOOP AFTER PROLONGED USE

Y Biale S Lazer and N Ben Aderet

From the Department of Obstetrics and Gynecology A Soroka Medical Center and Faculty of Medicine Ben Gurion University, Beersheba Israel

In order to confirm or refute the theory that is the need to replace IUD's every few years due to aging 26 Lippes Loops were tested. The sediment found on the devices was composed mainly of calcium carbonate. After removal of the sedimentation it was seen that there was some corrosion of the body of the IUD which caused a decrease in the IUD thickness. X-ray tests showed that an IUD which has been in use for a long period of time is more rigid, has less ability to stretch and breaks under less pressure. It seems that the current medical opinion stating that IUD's should be replaced every few years is valid.

Many advantages of the intrauterine contraceptive device (IUD) are the reasons for the spread of its use and its importance as a method of birth control. In its present varied forms it is normally made of plastic which occasionally breaks when the device is in position. This breakage can result in uterine pain, bleeding and difficulty in removal. As a result of these hazards, medical authorities concerned with family planning feel that there is a need to routinely replace an IUD every 3-4 years (3, 4).

In order to confirm this theory 26 IUD's of the Lippes Loop type have been tested. These were in the uterus for various periods of time up to a maximum of seven years.

The variables used for these tests were:

The composition of the sediment deposited on the devices.

Deformation of changes in thickness due to the length of time in use.

The amount of pressure required to break the devices.

METHODS AND RESULTS

Lippes Loops Size C which remained in the uterus for up to seven years were tested. Nine of the IUD's were broken within the uterine cavity. Removal of IUD's was performed either because of expulsion

of a piece of loop without symptoms or because of pain or irregular uterine bleeding. In the former group there were four women and in the latter 27 women. Amongst the group of 22 women 5 were found to have broken IUD's and in 2 IUD threads could not be seen but X-rays revealed the broken IUD's. In three cases the diagnosis of broken IUD was made during its removal. We do not think that the attempted removal caused the breakage since the IUD's were broken several times. In one case a woman was pregnant with a broken IUD.

Immediately after removal the loops were washed with ion free distilled water to free them from any adherent matter and then thoroughly air-dried. The solid incrustations formed on the Lippes Loops were extracted with dilute hydrochloric acid.

The devices were weighed before placing the IUD in hydrochloric acid and afterwards, thus the amount of the sediment was quantified. The CO₂ produced by the action of hydrochloric acid and the sediment was absorbed in a solution of calcium hydroxide and measured by titration.

The sediment upon several IUD's was scraped, weighed and burned into ash with oxygen added and the CO produced was measured. This represents the total carbon of the sediment. The total carbon consists of an organic carbon fraction and an inorganic fraction that is carbonate. The organic carbon fraction is determined by subtracting the CO₂ produced by the sediment in hydrochloric acid from the total CO₂ measured by burning the sediment. The amount of the various cations in the hydrochloric acid solution was estimated by atomic absorption Perkin Elmers spectrophotometer model 303.

Table 1 Composition of the Deposit as related to duration of IUD in utero (percentage of total weight)

No traces of aluminum, cobalt, copper, manganese and iron.

Duration in uterus (years)	Number of devices	Calcium carbonate	Magnesium	Sodium	Zinc
0.5	3	80.2	0.39	1.7	0.14
1	3	81.8	0.41	1.87	0.15
2	10	83.1	0.32	1.8	0.19
5	8	77.2	0.32	0	0.21
7	2	78.5	0.35	1.83	0.18

Table II Comparison of identical segments of broken and unbroken IUDs (thickness in millimeters)

Numbers of IUDs tested are given within parentheses

Order of Segment	New (3)	Unbroken		Broken	
		2 years (2)	5 years (2)	2 years (2)	5 years (3)
I	1.89	1.86	1.85	1.78	1.79
II	1.57	1.59	1.57	1.44	1.42
III	1.82	1.83	1.80	1.80	1.78
IV	1.57	1.57	1.52	1.42	1.39
V	1.83	1.82	1.80	1.75	1.73
VI	1.53	1.52	1.53	1.39	1.36
VII	1.72	1.73	1.70	1.67	1.69
VIII	1.44	1.43	1.43	1.40	1.38
IX	1.65	1.64	1.62	1.58	1.58

depression whereas a device which had been in use for 5.5 years would break when a pressure of 25 kg was applied (Fig. 3). The graphical presentation of such an experiment is given in Fig. 4.

DISCUSSION

The modern plastic IUD has been in use since the early 1960s and since then many side effects have been described such as abdominal pain, irregular uterine bleeding, pregnancies and expulsion of the device. The present paper reports on uncommon complication which has received little attention that is the fracture of the device *in situ*.

Fractures of plastic IUDs appear after retention in the uterine cavity for at least two years (1, 2, 4). Fragmentation occurs in 1–3% of insertions (1, 2) and the clinical manifestation is generally expulsion of a piece of loop without symptoms. Less frequently pain or irregular uterine bleeding occur necessitating hospitalization and anesthesia for removal of the IUD fragments. Fracture may affect the contraceptive action and an increased rate of pregnancies is attributed to broken IUDs (1, 5). A pregnancy associated with a broken IUD was observed also by us. Because of the above mentioned complications it is recommended to remove the fragmented loops (1) and some authors have adopted the practice of removing loops after two or more years and inserting fresh loops (3, 4).

The use of Lippes Loop is very popular and since relatively more information about its fragmentation is available (2, 4, 6) we decided to estimate the structural changes and the composition of the deposit on a Lippes Loop. After retention in the

uterus for some time IUDs develop a rough porous surface due to depositions of calcium salts and the extent of the deposit appeared to be related to the time the IUD had been retained (6, 7, 8). Engineer et al (8) had observed that the various constituents including protein accounted for about 70% of the total solid incrustation present on a device and that the chemical nature of the remaining 30% of the material could not be elucidated. We found that the inorganic portion is greater composed mainly of calcium carbonate (around 80%) and about 20% organic material. Also the magnesium level was found by us to be much lower i.e. 0.37% after compared with the 10% found in their work. We demonstrated the presence of 1% sodium and 0.15% zinc while Engineer et al (8) did not find these cations. Neither Engineer et al nor we could demonstrate traces of cobalt or copper. It is interesting to note that Kar et al (9) reported that calcium was slightly increased and bicarbonate slightly decreased in the intrauterine fluid of IUD fitted patients. The rough solid deposit on the IUD is not responsible for bleeding by an abrasive action on the endometrium (7).

Another interesting observation is the erosion of the body of the devices with the appearance of moth eaten cloth. The extent of the corrosion increased in proportion to the length of time that the device had been used. The corrosion caused a decrease in the IUD thickness in those devices which had been broken. These changes may be related to the chronic foreign body reaction by the endometrium to the presence of an IUD. It has been shown that polymorphonuclear leukocytes and mononuclear cells are present more frequently and in increased numbers in uterine flushings of animals with IUDs. The total protein reducing sugar and hemoglobinase values are also higher (10, 11, 12).

Our findings confirm the observation made by others (4, 6) that the length of time in use causes an increase in rigidity and decrease in flexibility of the devices. As a result the longer a device is in the uterine cavity the less susceptible it becomes to deform and breaks under less pressure. Naturally the IUD tends to break at the curved areas. This is evidently due to fatigue caused by deformation under uterine contraction. In addition the study showed that the structural thickness decreases when the devices were in use for a long time.

It is clear that comprehensive results and deductions can only be obtained by expanding the study.

s and the amount of testing. Nevertheless it has been demonstrated that the current medical opinion stating that IUD should be replaced every 5 years is a valid theory.

REFERENCES

- Obal M W & Vittoz Meynard Y. Intrauterine devices broken in situ. *Contraception* 2: 407 1970
- East M A. Fracture of the Lippes Loop in utero. *J Obstet Gynecol Br Comm* 79: 190 1972
- Yata T T. Textbook of Ota Ring. Tokyo Research Institute of Ota Ring
- Lang J E. Fracture of Lippes Loop. *Br Med J* 4: 795 1977
- Jomany Z. & Hansok M. Fractured Lippes Loop and pregnancy. *Br Med J* 1: 549 1973
- Yang T S & Yang W H. Calcium carbonate deposition on intrauterine contraceptive devices. *Am J Obstet Gynecol* 109: 664 1971
- Howard C. The significance of calcium deposits occurring on intrauterine devices. *J Obstet Gynecol Br Comm* 78: 861 1971
- Engineer A D, Dug Gupta P M & Karr A M. Chemical composition of the deposit formed on the Lippes Loop after prolonged use. *Am J Obstet Gynecol* 106: 315 1970
- Kar A B, Engineer A M, Gaei R, Kamboj V M, Dug Gupta R R & Chowdury S R. Effect of an intrauterine contraceptive device on biochemical composition of uterine fluid. *Am J Obstet Gynecol* 101: 966 1968
- Peplow W, Breed W G, Jones M J & Eckstein P. Studies on uterine flushings in the baboon. Part I. *Am J Obstet Gynecol* 116: 771 1973
- Peplow W, Breed W G & Eckstein P. Studies of uterine flushings in the baboon. Part II. *Am J Obstet Gynecol* 176: 780 1973
- Moyer D L & Mishell D R. Reactions of human endometrium to the intrauterine foreign body. *Am J Obstet Gynecol* 111: 66 1971

Submitted for publication July 1 1977

Y Biale

Department of Obstetrics and Gynecology A
Soroka Medical Center
Beersheba
Israel

INFLUENCE OF PUBOCOCCYGEAL REPAIR ON URETHRAL CLOSURE PRESSURE AT STRESS

Görel Bunne and Anders Öbrink

From the Department of Obstetrics and Gynecology, Karolinska Institutet, Sabbatsberg Hospital, Stockholm, Sweden

Stress incontinence is cured or improved by treatment but the immediate reason is obscure. Simultaneous urethrocytometry with urethral pressure recording at rest pre- and postoperatively has shown that the urethral pressure remains fairly unchanged. Similar measurements but during the operation have been performed in eight women with stress incontinence before and after pubococcygeal repair with focus on changes in pressure transmission from abdomen to urethra. We have found that the reason for the greatly improved pressure transmission is probably depending on the firm support beneath the urethra postoperatively. Rotational descent is prevented by the "floor" beneath the urethra responds with good urethral pressure at stress. The pressure transmission was studied at one year as at one month after surgery pointing out the long-term result.

Pubococcygeal repair, a well known and much used operation for stress incontinence, was introduced by Ingelman Sundberg in 1947 (3). Several retrospective studies have shown that it is suitable for treating severe degrees of stress incontinence (4-6). Measurements at rest in the urethra and in the bladder before, during and after the operation have been investigated by means of simultaneous urethrocytometry (7). The only postoperative change in the urethral pressure profile (=the intraluminal pressure recorded continuously through the entire length of the urethra) was a broader distribution of maximal pressure in place of the distally located point of maximal pressure found to be typical of stress incontinence. This alone cannot account for the transformation from stress incontinence to continence although it might be of marginal importance.

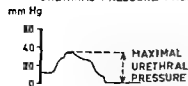
We have found that the main determinant of the degree of continence or stress incontinence is the magnitude of the urethral closure pressure at rest reflecting the tone of the smooth and striated muscles in the urethral wall and in its surroundings including the supporting tissues (2).

Pubococcygeal repair did not make any contribution to the maximal urethral pressure. There was on the contrary a tendency to a lowered pressure possibly reflecting damage to the urethral blood supply in connection with the vaginal dissection (7). Thus the reason for cure could not be concluded from measurements at rest. Most likely improvement arose from a better transmission of pressure from abdomen to urethra. To verify this, eight patients were examined both pre- and postoperatively in a dynamic situation simulating the efforts of daily life by coughs of different strength. The equipment used for simultaneous urethrocytometry was elaborated in 1974 (1) and gives a good precision and reproducibility even at high frequencies and amplitudes. Dynamic studies are made feasible by the use of a thin, semiflexible catheter containing the pressure measuring areas. Accordingly, the results of the operation can be evaluated not only from the patient's history but also objectively by determining the exact margin to leakage at different degrees of straining. Among other things, pressure transmission from abdomen to urethra can be calculated. To further evaluate any improvement in this respect, six women have been examined in the same manner one year postoperatively.

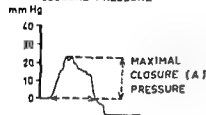
MATERIAL AND METHOD

Eight women, mean age 57 years ($r=69-43$), mean parity 0 ($r=3-1$) with genuine stress incontinence of second degree on the Ingelman Sundberg scale (5) were examined pre- and one month postoperatively. No patient had been operated on previously and all had a marked downward rotation of the anterior vaginal wall including the bladder neck area at straining. A Bonney positive urinary leakage could be provoked. All were similarly treated surgically with pubococcygeal repair as described by Ingelman Sundberg. This involves an arcuate incision at introus dissection of the anterior vaginal wall, sutur-

URETHRAL PRESSURE PROFILE AT REST



CLOSURE PRESSURE



BLADDER PRESSURE

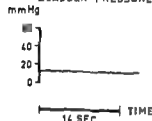


Fig 1 The three simultaneously recorded parameters

ing the pelvic fascia in the midline to elevate the bladder and bladder neck, cutting the pubococcygeal muscle bilaterally and using the anterior portions for a sling to support the proximal urethra. The distal urethra supported by part of the two bulbocavernosus muscles sutured together in midline.

The equipment used for simultaneous urethrocystometry consists of a thin (diam 3.1 mm) flexible Dacron catheter with two sensor areas, at its tip and another 80 mm proximally. Three amplifiers and a recorder. Electronic subtraction gives the closure pressure (=urethral pressure minus bladder pressure). To record the urethral pressure profile, the catheter was fed by a motor through the urethra at a velocity of 1 cm/s.

In the lithotomy position the urethral pressure profile at rest was recorded at bladder volumes of 100 and 300 cc. Each volume, cough profiles during light (approx. 10 mmHg), moderate (approx. 30 mmHg) and strong (approx. 50 mmHg) coughs were also recorded. The subjects coughed repeatedly, approximately once a second, with constant force while the profile was registered. If the urethral wall comes into direct contact with the sensor areas (as when the urethra is kinked or extremely narrow), artefacts may render interpretation of the profile somewhat difficult. Consequently these measurements were supplemented with another estimation of the degree of pressure transmission. This was obtained by holding the catheter manually with the proximal sensor area at a point of highest urethral pressure while the patient coughed repeatedly as described above.

Six women, mean age 54 years (range 49-63) and mean weight 62.5 (4-1) were examined in the same way one year

URETHRAL PRESSURE PROFILE DURING REPEATED COUGHS

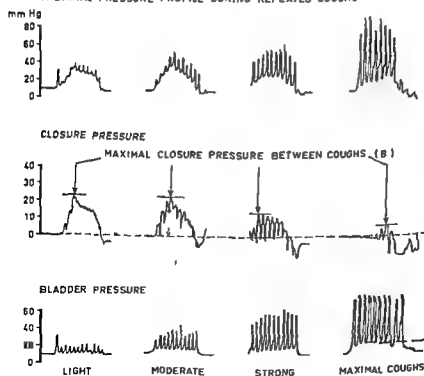


Fig 2 The same parameters as in Fig 1 during repeated coughs

occygeal repair For this investigation only the de
of pressure transmission was calculated

DEFINITIONS AND INTERPRETATIONS

re The pressure within the urethra in rela
to the atmospheric pressure

r vesical pressure The pressure within the bladder
ation to the atmospheric pressure

thral pressure profile Continuous recording of the
uminal pressure throughout the entire length of the

ra thral closure pressure The intraurethral pressure
the bladder pressure (At rest=A)

functional length of the urethra The part of the
re where the intraluminal pressure exceeds the blad
pressure

urethral maximal closure pressure between repeated
s.

dynamic urethral maximal closure pressure (at the
nt of a cough)

B signifies the loss of urethral closure pressure im
ately after repeated coughs This reduction can be
me in part by a defective transmission of abdominal

II during the recording of the urethral cough profile
nly by a loss of urethral pressure which will be

esed below

e the degree of straining (mmHg) calculated from the
er pressure curve at the time of maximal dynamic

re pressure

C signifies the defect in pressure transmission to
at a cough (The bladder pressure can be supposed

lect the intra abdominal pressure)

C the total loss of urethral closure pressure at the
nt of cough

(B+S) signifies the minimal loss of urethral closure
re that is not attributable to defective transmission

un between coughs

Figs 1 and 2

RESULTS

Patients were subjectively cured by the opera

The bladder neck was well elevated no

ficant rotational descent occurred at straining

the muscle sling had contractile ability No

ry retention was seen postoperatively except

case (residual volume of 150 ml)

seen from table I the functional length and

bladder pressure remained unchanged after

ry while the maximal urethral pressure

ed slightly deteriorated

he obvious improvement in pressure transmis

is shown in table II (see B-C) Four fifths of

intraabdominal pressure increase were trans

ed to the urethra postoperatively diminishing

and thus increasing C the margin to leakage

Table I Values at rest

n=8 The data are mean values with the standard devia
tion in parenthesis

Bladder content ml	Before surgery		After surgery	
	100	300	100	300
Functional length (mm)	25.4 (4.7)	4.3 (5.6)	25.6 (3.9)	25.8 (3.5)
Max closure pre sure (mmHg)	27.1 (2.8)	7.4 (3.1)	17.3 (6.6)	15.3 (4.4)
Bladder pressure (mmHg)	10.3 (3.5)	10.9 (7.8)	9.8 (7.9)	9.9 (1.8)

Student's *t* test has been used for the statistical analysis
Statistically significant differences before and after opera
tion are italicized ($p < 0.05$)

However C was not as great as expected consider
ing the constantly improved pressure transmission
This is explained by the slight reduction in closure
pressure at rest which was brought about by the
operation

DISCUSSION

No divergences in the pressures at rest were seen
compared with previous investigations of the re
sults of pubococcygeal repair (7) The short func
tional length typical of stress incontinence
persisted The tendency to a lowered urethral clo
sure pressure noticed earlier was verified Obvious
ly the impaired vascularization after extensive vag
inal dissection causes a reduction in urethral pres
sure by influencing the tone of the urethral smooth
and striated muscles If the initial preoperative
urethral maximal pressure is very low it is espe
cially important to avoid further reduction and
hesitation about the benefit of a vaginal operation is
justified On the other hand it was clear that this
reduction in urethral pressure could be made up for
by the greatly improved pressure transmission of
the urethra accomplished by pubococcygeal repair

Both in continence and in stress incontinence the
loss of pressure on the way from abdomen to
urethra amounts to 35-40% of the pressure increase
in question (2) this may be compared with approx
imately 20% after pubococcygeal repair A much
better transmission than ever seen in middle aged
continent women had been accomplished Of the
intra abdominal pressure increase at any effort
80% reached the most effective part of the func
tional urethra this was enough to maintain conti

Table II Values during coughing (mmHg)

The data are mean values with the standard deviation in parenthesis

Bladder content ml	Before surgery		After surgery	
	100	300	100	300
<i>Light coughs (approx 15 mmHg)</i>				
A	22.1	21.4	17.3	15.3
B	19.8 (4.71)	17.3 (4.74)	16.3 (6.52)	13.4 (4.81)
C	16.5 (4.57)	13.9 (5.03)	12.9 (6.47)	10.5 (5.48)
B-C	3.3 (0.46)	3.4 (0.74)	3.4 (1.30)	2.9 (0.99)
A-(B+S)	0	1.9 (2.89)	0	0
A-C	5.6	7.5	4.4	4.8
<i>Moderate coughs (approx 30 mmHg)</i>				
A	22.1	21.4	17.3	14.3
B	16.6 (4.24)	14.5 (5.76)	14.6 (7.29)	13.1 (5.91)
	3.6 (5.50)	3.4 (5.76)	8.5 (7.63)	7.6 (6.14)
B-C	13.0 (3.87)	10.9 (2.42)	6.1 (1.46)	5.5 (0.76)
A-(B+S)	3.4 (4.17)	3.0 (6.05)	0.1 (1.85)	0
A-C	18.5	18.0	8.8	7.7
<i>Strong coughs (approx 50 mmHg)</i>				
A	22.1	21.4	17.3	15.3
B	14.9 (3.67)	10.7 (4.63)	12.8 (5.85)	10.5 (5.04)
C	7.2 (3.53)	8.1 (4.34)	3.5 (5.93)	2.4 (5.34)
B-C	18.4 (3.95)	19.0 (3.27)	10.5 (4.38)	8.1 (1.96)
A-(B+S)	5.0 (6.27)	7.7 (5.55)	1.6 (1.97)	1.5 (2.97)
A-C	24.2	29.5	13.8	12.9

Student's *t* test has been used for the statistical analysis. Statistically significant differences before and after operation are italicized ($p < 0.05$).

nence even with strong coughs in spite of the low maximal closure pressure at rest.

The sutured pelvic fascia and above all the muscle sling create a firm floor under the urethra keeping it in a normal or slightly elevated position even during intense straining. Downward rotation is prevented almost entirely. The counterpressure exerted by this floor is certainly much better than

Table III Transmission after 1 year (mmHg)

n=6

Bladder content ml	100	300
B-C light coughs	7.0 (0)	2.3 (0.4)
B-C moderate coughs	6.3 (7.50)	6.3 (1.67)
B-C strong coughs	8.8 (7.49)	9.5 (3.2)

that produced by a sagging anterior vaginal wall. We believe that the support for the urethra is more important than elevation in itself, although technically elevation is a condition for achieving a support. Bonney's test, for example, elevates the urethra but also prevents downward rotation by straining by support from the examiner's finger or an instrument. As soon as rotational descent is allowed, leakage appears in stress incontinence.

One year postoperatively pressure transmission was found to be equally good, indicating that relaxation or destruction of the urethral support had not occurred. In a follow-up (6) the major recurrence after pubococcygeal repair occurred during the first postoperative year, often before healing was complete. Most likely they are due to rupture of the muscle sling, deprived of its solid floor, the urethra yields to a sudden strain and pressure transmission is again as defective as in stress incontinence. If the muscle sling is still effective after one year, the long-term prognosis should be good.

CONCLUSION

Pubococcygeal repair ad modum Spiegelthal and Berg cures stress incontinence by firming of the urethra with a solid floor, thereby preventing rotation and descent at straining and resulting in an improved transmission of pressure from abdominal urethra. Losses are reduced to approximately 10% which converts stress incontinence into continence. The improvement is maintained during the first postoperative year (=the observation time) probably much longer.

REFERENCES

1. Asmussen M & Ulmsten U. Simultaneous urethrocystometry with a new technique. *Scand J Urol Nephrol* 10: 7, 1976.

— Öbrink A. Urethral closure pressure at rest—a comparison between stress incontinent and continent women. Accepted for publication in *Urology Research* 1977.

— Sundberg A. Extra vaginal plastic repair of pelvic floor for prolapse of the bladder neck: a new method to operate for stress incontinence. *Gynaecologia* 173: 147, 1947.

— Ingelman Sundberg A. Urinary incontinence in women excluding fistulas. *Acta Obstet Gynecol Scand* 256: 195.

— Ingelman Sundberg A. Urininkontinens hos kvinnan. *Läk Med* 50: 1149, 1953.

— Öbrink A. Pubococcygeal repair ad modum Ingelman Sundberg. A retrospective investigation with

10–15 years time of observation. *Acta Obstet Gynecol Scand* 56: 391, 1977.

7 Öbrink A, Bunne G, Ulmsten U & Ingelman Sundberg A. Urethral pressure profile before, during and after pubococcygeal repair for stress incontinence. *Acta Obstet Gynecol Scand* 57: 49, 1978.

Submitted for publication May 19, 1977

Görel Bunne
Department of Obstetrics and Gynecology
Sabbatsberg Hospital
Stockholm
Sweden

CRYOSURGICAL TREATMENT OF DYSPLASIA AND CARCINOMA IN SITU OF THE CERVIX UTERI

Yngve Einert

From the Department of Obstetrics and Gynaecology, Helsingborg Hospital, Helsingborg, Sweden

Fifty nine patients (age range 18-40 years, mean 31) with dysplasia or carcinoma in situ of the cervix were treated with cryosurgery. Healing occurred in 48 (81.6%) after one treatment (double freeze-thaw) and in 7 patients after refreezing. No early or late complications occurred during a follow-up period of 7-37 months. Five of the patients with carcinoma in situ were tested immunologically with determinations of serum (tissue polypeptide antigen) which can be employed as a marker of malignancy. In all instances the initially elevated TPA normalized after cryosurgical treatment. Cryosurgery is a simple, painless and safe treatment modality for ectocervically localized carcinoma in situ and dysplasia, and is particularly suited for out-patient use.

The routine use of vaginal cytology has resulted in an increased number of diagnosed cases of carcinoma in situ. In 1970 the number of diagnosed cases of in situ cervical cancer in Sweden was according to the official Swedish Cancer Register 11, more than 4000 (26). The predominant treatment of carcinoma in situ is surgical conization (17, 18). In more recent years, however, the number of conizations performed has decreased, mainly owing to the introduction of alternative treatment methods, e.g. cone biopsy (20). Conization gives a healing frequency in excess of 95% but necessitates hospitalization and is attended by various complications, viz. post-operative hemorrhage varying in frequency between 6.6 and 11% in different studies (8, 10, 15, 16), cervical stenosis in 4 to 6.6% (10, 15, 17) and also probably a slight increase in the frequency of spontaneous abortion, premature delivery and complications during childbirth, although there are some contradictions in the published works (10, 17). During the last decade, cryosurgery has been regarded as an alternative to surgical conization of malignant conditions of the uterine cervix (5, 6, 22, 23, 24, 25). The indication for cryosurgery has been limited to discrete, colposcopically visi-

ble lesions on the ectocervix as the diagnosis and cryosurgical management of endocervical lesions has been found to be unsatisfactory (5, 24, 25).

The healing rate after cryosurgery varies between 81 and 97%. There are few complications; a few cases of salpingitis have been reported, but there have not been any confirmed occurrences of cervical stenosis or any untoward effects with respect to fertility or pregnancy (5, 14, 22, 23, 24, 25).

Besides the immediate tissue necrotizing effect of the cryosurgical procedure, there is also some evidence for a late induced immunological effect. There is a rise in the titre of different tumour antibodies after cryosurgery (12, 21).

In these investigations, there was a greater increase in titre than after conventional surgery or radiotherapy, and even greater than the antibody response that is obtained after the injection of tumour extract.

MATERIAL AND METHODS

Women were selected who had had two or three vaginal smears (VS) showing abnormal cells consistent with dysplasia or carcinoma in situ. Cervical curettage and a colposcopically selected biopsy of the portio vaginalis were performed. The colposcopic evaluation of the cervix showed in most cases areas of white epithelium and abnormal vascular pattern, referred to as mosaic or punctuation (5, 11) and was used for identifying areas to be biopsied. No treatment was given and after 3 months further cytological smears were taken. This was done because of colposcopically selected biopsies have been proved to be curative in up to 50% of cases (1).

Three patients with endocervically localized lesions and 1 patient with micro-invasive cancer were identified and given other forms of treatment. In 39 patients the biopsy was curative. Thus 59 patients remained who had persisting dysplasia or carcinoma in situ; these women were given cryosurgical treatment (Fig. 1).

Freezing was performed with the MT 500 Multitup Gyn Cryosurgical Probe, manufactured by Cryomedics Inc., Bridgeport, Connecticut, USA. Applicators of differ-

without anaesthesia moreover as a rule there is no post operative incapacity

In the present investigation the agent employed was carbon dioxide which gives a temperature of about -70°C . In other investigations as well as carbon dioxide nitrous oxide and freon have been used which provide a temperature of about -90°C as well as liquid nitrogen which yields a temperature of -190°C . No controlled clinical study has been published to date in which the effects of different refrigerant agents have been compared. It is very likely that the treatment period can be shortened and perhaps the procedure can be simplified by using gases yielding a lower temperature but the most important prerequisite is that the rate of temperature fall exceeds 5°C per sec. this is obtained even with carbon dioxide (12). Freezing in two sessions with a short thawing period in between has also proved to give better results than a single session (5, 9).

The results of the present investigation accord with earlier published studies (5, 14, 22, 23, 24, 25) in the absence of early and late complications. The present results do not suggest that a previously contracted salpingitis or an in situ intra uterine device should constitute a contra indication.

It is considered important to perform the cryosurgery in the early proliferative phase of the uterine cycle because the appreciable cervical oedema that the treatment engenders during the first post operative week might cause temporary cervical obstruction and consequently a risk of haematometra. There have not been however any observed untoward effects as regards fertility or pregnancy nor have there been any confirmed occurrences of cervical stenosis. These findings make the method a suitable one especially for women that desire to have further children.

A careful follow up like that conducted after surgical conization is essential. Refreezing can be performed in cases of persistent dysplasia and the possibility always remains open for surgical conization. There have not been any recurrences of dysplasia or carcinoma in situ in this study but the observation period is still rather short. Crisp (6) has however followed 60 patients cryosurgically treated for severe dysplasia for 6-7 years and has not found any recurrences.

In 1957 Björklund et al. (2) discovered and purified a polypeptide with antigenic properties. Its tissue polypeptide antigen (TPA). This antigen occurs

in tumour tissue and pathologically elevated TPA activity in the serum can be demonstrated in some patients with a malignant disease (3, 7, 19). There is a correlation between remission in the treatment of malignant tumours and the return to normal of TPA activity as well as between rising TPA activity and progressive tumour disease (4). In a study with carcinoma in situ in the present investigation that initially had a slightly pathologically elevated TPA activity in the serum levels fall to normal after cryosurgical treatment. All 5 patients were also completely healed TPA activity may be a useful sign of successful treatment.

CONCLUSIONS

Cryosurgery is a rapid non anaesthetic method of treatment that can be used in dysplasia and carcinoma in situ localized and limited to the ectocervix. It is above all suitable for young groups. The method places high demands on a direct pre operative diagnosis. The treatment can be performed on an out patient basis and there is as a rule no post operative incapacity. There has been complete remission in more than 90% of the patients that were treated. The method is not accompanied by any early or late complications and does it appear in any way to adversely affect fertility or pregnancy.

REFERENCES

1. Ahlgren M, Lindberg L-G & Nordquist S. Management of carcinoma in situ of uterine cervix. Colposcopically selected local excision. 19th Ann Congress on Obstet Gynecol. Reykjavik 1974. In press.
2. Björklund B & Björklund V. Antibody of polypeptide human malignant and normal tissues by a immunological technique. presence of an immunizable tumor antigen. *Int. Arch. Allergy* 1957; 10: 1-10.
3. Björklund B, Björklund V, Wiklund B, Liljeström M, Ekdahl M, Hagbard L, Kanner Eklund G & Luning B. A human tissue polypeptide related to cancer and placenta. I. Preparation and properties. II. Assay technique. III. Clinical studies. 148 individuals with cancer and other conditions. In: *Immunological Techniques for Detection of Cancer* (ed. Björklund), p. 133. Bonnier's Stockholm 1971.
4. Borgstrom M & Maltson W. In: *Third Annual Symp. on Detection and Prevention of Cancer*. New York 1976. In press.
5. Creasman W T, Townsend D E, Horland D

- 1 Upton III T Colposcopy and cryosurgical treatment of severe cervical intraepithelial neoplasia. *Obstet Gynecol* 41: 501 1973
- 2 Nsp W E Cryosurgical treatment of neoplasia of the uterine cervix. *Obstet Gynecol* 39: 495 1977
- 3 Jernth Y Tissue polypeptide antigen (TPA) and preinvasive stages of cervical cancer. 19th Nordic Congress on Obstet Gynecol Reykjavik 1976. In press
- 4 Nilsson B, Lundgren B & Norden J Carcinoma in situ cervicis uteri. *Läkartidningen* 61: 1974 1964
- 5 Hill W, Fraser J D & Carter D C Repeated freeze thaw cycles in cryosurgery. *Nature (Lond)* 19: 410 1968
- 6 Jellström I Konisation—behandling av preinvasiv cervixcancer [Konization treatment of preinvasive cervical cancer]. *Läkartidningen* 71: 1739 1974
- 7 Jellström V E & Chanen W The use of colposcopy in the selection of patients for cervix cone biopsy. *Am J Obstet Gynecol* 114: 185 197
- 8 Jellström H B & Sander S Cryosurgery: its scientific bases and clinical application. *Practitioner* 219: 543 1973
- 9 Hülka H S Punch biopsy and konization as diagnostic procedures after abnormal cervical smears. *Obstet Gynecol* 36: 54 1970
- 10 Kaufman R H & Connor J S Cryosurgical treatment of cervical dysplasia. *Am J Obstet Gynecol* 108: 1167 1971
- 11 Kim A-C Konisation vid cancer in situ [Konization in cases of cancer in situ]. *Läkartidningen* 57: 1519 1970
- 12 Kottmeier H L Synpunkter på cancer coli uteri stadium I och dess behandling. In: *Obstetrik och gynecologi. Nya forskningar och rön* (ed C Gemzell) del 1 pp 140 Almqvist & Wiksell Stockholm 1967
- 13 Kullander S & Sjöberg N O Behandling av carcinoma in situ cervicis uteri med konisering [The treatment of carcinoma in situ cervicis uteri by conization]. *Läkartidningen* 68: 3398 1971
- 14 McLaren H C Management of cervical carcinoma in situ. *Lancet* ii: 683 1968
- 15 Menendes Botet C J & Schwartz M K Evaluation of tissue polypeptide antigen (TPA) in serum and/or urine of patients with cancer or benign disease. 170th National Meeting of the American Chemical Society Division of Biological Chemistry Chicago Ill Aug 24-29 Abstract 1975
- 16 Segerbrand E & Lundström P Ringbiopsi/konisation—presentation av ett material. *Obstetrik och gynecologi* 1975 p 30
- 17 Shulman S In: *Cryosurgery* (ed R W Rand A P Rinfret & H von Leden) pp 78 Charles Thomas Springfield Ill 1968
- 18 Stendahl U Cryosurgery in dysplasia and cancer in situ cervicis uteri. 19th Nordic Congress on Obstet Gynecol Reykjavik 1976. In press
- 19 Stendahl U & Stenson S Cryokirurgi vid dysplasi och carcinoma in situ. *Kliniska erfarenheter [Cryosurgical treatment of dysplasia and carcinoma in situ]*. *Läkartidningen* 71: 2933 1974
- 20 Townsend D E Cryosurgery techniques symposium. *Contemp Obstet Gynecol* vol 5 May 1975
- 21 Townsend D E, Ostergaard D R & Hirose F The effect of cryosurgery on the cervix uteri. In: *Cryosurgery symposium* Los Angeles 1967 (ed R W Rand A P Rinfret & H von Leden) pp 377 Charles Thomas Springfield Ill 1968
- 22 Cancer incidence in Sweden 1970. *Socialstyrelsens cancerregister* 1973

Submitted for publication April 27 1977

Yngve Einert
Lakargruppen
Södergatan 39
S 252 25 Helsingborg
Sweden

SHORT COMMUNICATIONS

HUMAN PLACENTAL LACTOGEN LEVELS IN AMNIOTIC FLUID
IN NORMAL AND TOXEMIC PREGNANCIES

D. Lolis and M. Kaskarelis

*From the First Department of Obstetrics and Gynecology, University of Athens
Alexandra Maternity Hospital, Greece*

Abstract Amniotic fluid human placental lactogen (HPL) levels were measured by radioimmunoassay in 162 cases of women with normal pregnancy and 43 with toxemic pregnancy in the last trimester of pregnancy. A significant difference in levels was observed.

Previous investigations have measured human placental lactogen levels in the amniotic fluid in only a few cases of both normal pregnancies and pregnancies complicated by Rhesus isoimmunization. However, nothing has been reported for measurements of HPL in amniotic fluid in toxemic pregnancies (1-8, 14, 23).

The present study was to investigate the value of variations of HPL levels in amniotic fluid at various weeks of normal and toxemic pregnancies in order to evaluate the fetal distress.

All the samples were put in plastic tubes with code Nos. and sent to the laboratory, where the origin of sample (i.e. from normal or toxemic pregnancy) was unknown.

They were then centrifuged and the serum stored at -20°C until completion of the calculation of hormone by radioimmunoassay. In the present study the Amersham HPL kit was used for determination of HPL level in amniotic fluid.

RESULTS

The results of investigation are presented in Table I. A significant difference of HPL values was observed between normal and toxemic pregnancies, even though it was observed that the values rose both in normal and toxemic pregnancies until the 34th-36th week. Thereafter there was a decline (Fig. 1).

MATERIAL AND METHOD

A total of 205 amniotic fluid samples were collected from 162 women with normal pregnancy and from 43 women with toxemic pregnancy between the 28th and 42nd week of gestation. These cases were classified into four groups according to the age of gestation as follows:

- Group A: 28th to 33rd week: 33 cases
- Group B: 34th to 36th week: 31 cases
- Group C: 37th to 39th week: 55 cases
- Group D: 40th to 42nd week: 44 cases

The amniotic fluid samples were taken by amniocentesis when the cervical dilatation was no more than 5 cm.

DISCUSSION

In recent years the study of the hormones and enzymes of amniotic fluid have been started. It can be supported that the determination of amniotic fluid's hormones is useful for the estimation of the endocrine status of the fetus (15).

Many investigations have been published about HPL levels in the maternal blood in normal and abnormal pregnancies as a means of observing the condition of the fetus, but there are limitations in

Table I. Mean values of the amniotic fluid HPL ($\mu\text{g/ml}$) in normal and toxemic pregnancies

Age groups in gestational weeks	Normal pregnancies				Toxemic pregnancies				
	n	X	S.D.	S.E.	n	X	S.D.	S.E.	t
A 28-33	33	0.78	0.430	0.100	15	0.50	0.00	0.004	2.800
B 34-36	19	0.85	0.356	0.081	17	0.59	0.00	0.050	3.09
C 37-39	45	0.77	0.400	0.050	10	0.41	0.238	0.075	3.563
D 40-42	80	0.64	0.072	0.002	6	0.17	0.148	0.060	7.833

$P < 0.01$ $P < 0.001$

G M L P E G CES
 — TO EMIC PREGNANCIES
 1

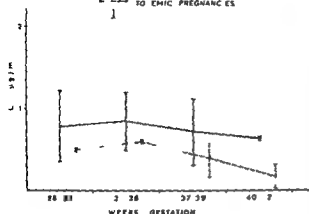


Fig. 1 Mean values and standard deviations of amniotic fluid HPL in normal and toxemic pregnancies

vestigations about the fluctuations of HPL in amniotic fluid (2 5 8 9 12 14 16 17 19 23)

In this study determinations of HPL levels were made in amniotic fluid in a large number of normal and toxemic pregnancies. The amniotic fluid samples were taken at the beginning of labour in order to avoid the influence of labour in HPL levels (8). It was found that the HPL levels rose reaching the highest value between the 34–36 week of pregnancy. Thereafter there was a decline. Our findings that the HPL levels rose with progress of pregnancy agree with findings of other investigators but there are different opinions as to which week of pregnancy the HPL levels stop rising (1 3 6 7 10 11 18 20 21 22).

It was noticed that the HPL levels of amniotic fluid of toxemic pregnancies are much lower than in normal pregnancies at the same week. It is not possible to compare our findings with others because this has never been done before by other investigators.

How the HPL is carried from the blood circulation into the amniotic fluid is unexplained (4). According to the opinion of Josimovich et al (10) the HPL passes through the placenta into amniotic fluid from the membranes by diffusion but it is necessary to carry out further investigations in order to fully explain this problem (13).

In conclusion the present study showed that measurement of HPL of the amniotic fluid is a good parameter for the estimation of fetal risk in cases with toxemia. Even one sample may help in the diagnosis of impending fetal distress or be an indication of fetal death.

REFERENCES

- 1 Beck P, Parker M & Daughaday W. Placental lactogen in plasma during normal pregnancy and pregnancy with gestational diabetes. *Clin Res* 13: 1965.
- 2 Berle P. Human placental lactogen in amniotic fluid during normal and pathologic pregnancies. VII World Congress of Obstetrics and Gynecology, Moscow 12–18 August 1973.
- 3 Chard T. HPL Levels in a Guide of Foetal Well-being during Pregnancy. The Radiochemical Centre, London 1973.
- 4 Chez R, Josimovich J & Schultz S. The transfer of human placental lactogen across isolated amnion. *Choenon Gynecol Invest* 1: 312 1970.
- 5 Cohen M, Haour F, Bertrand J & Dumont M. L'hormone lactogénique placentaire (HPL) ou nouvelle hormone chorionique. *Gynecol Obstet (Paris)* 69: 197 1970.
- 6 El Tomi A E, Crystle C H & Stevens V. Plasma human placental lactogen in late pregnancy at labour. *Am J Obstet Gynecol* 108: 345 1970.
- 7 Gaspard U & Franchimont P. Evolution des taux de l'hormone chorionique somatomammotrope (HCS) au cours des grossesses à haut risque. XXIV Congrès de la Fédération des Sociétés de Gynécologie et Obstétrique de la langue Française, Tunis 17–15 Sept. 1977, p. 324.
- 8 Grumbach M M, Kaplan S L, Sciarra J J & Burr I M. Chorionic growth hormone prolactin (CGP). Secretion, disposition, biologic activity in man and postulated function as the "growth hormone" of the second half of pregnancy. *Ann NY Acad Sci* 148: 501 1968.
- 9 Hechterman R, L. Hermite, Balenaut M, Delvaux C, Jacob D & Flamme P. Dosage radio-immunologique du HPL dans le liquide amniotique prélevé par amniocentèse au voisinage du terme de la gestation. XXIV Congrès de la Fédération des Sociétés de Gynécologie et Obstétrique de la langue Française, Tunis 17–15 Sept. 1977.
- 10 Josimovich J B, Kosor B, Boccella L, Metz D H & Hutchinson M L. Placental lactogen in maternal serum as an index of fetal health. *Obstet Gynecol* 36: 244 1970.
- 11 Kaplan S & Grumbach M. Immunoassay for human chorionic growth hormone prolactin in serum and urine. *Science* 147: 751 1965.
- 12 Leitchworth A T, Boardman M, Brivona C, Landon J & Chard T. A rapid semi-automated method for the measurement of human chorionic somatomammotrophin. The normal range in the third trimester and its relation to fetal weight. *J Obstet Gynecol Br Commonw* 78: 447 1971.
- 13 Moser H J & Hollingsworth D M. Radioimmunoassay of human chorionic somatomammotrophin in serum, amniotic fluid and urine. *Clin Chem* 19: 607 1973.
- 14 Niven P A R, Werd R H T & Chard T. Human placental lactogen levels in amniotic fluid in Rhoeus immunization. *J Obstet Gynaecol Br Commonw* 81: 988 1974.

- 6 Rajan R. Amniotic fluid assays in high risk pregnancy. *Clin Obstet Gynecol* 16: 313, 1973.
- 7 Samaan N, Yen S, Friesen H & Pearson O. Serum placental lactogen levels during pregnancy and in trophoblastic disease. *J Clin Endocrinol* 26: 1303, 1966.
- 8 Seppala M & Ruoslahti E. Serum concentration of human placental lactogenic hormone (HPL) in pregnancy complications. *Acta Obstet Gynecol Scand* 49: 143, 1970.
- 9 Soger W, Desjardins P & Enesen H. Human placental lactogen: An index of placental function. *Obstet Gynecol* 36: 777, 1970.
- 10 Spellacy W, Cohen W, Carlson K. Human placental lactogen levels as a measure of placental function. *Am J Obstet Gynecol* 97: 560, 1967.
- 11 Spellacy W. Human placental lactogen in high risk pregnancy. *Clin Obstet Gynecol* 16: 298, 1973.
- 12 Spona J & Janisch H. Serum placental lactogen (HPL) as an index of placental function. *Acta Endocrinol* 68: 401, 1971.
- 13 Teoh E, Spellacy W & Buhi W. Human chorionic somatomammotrophin (HCS): A new index of placental function. *J Obstet Gynaecol Br Comm* 78: 673, 1971.
- 14 Tyson J, Hwang P, Guyda H & Friesen H. Studies of prolactin secretion in human pregnancy. *Am J Obstet Gynecol* 113: 14, 1972.

Submitted for publication Dec 16 1976

D Lolis

First Department of Obstetrics and Gynecology
University of Athens
Alexandra Maternity Hospital
80 Vass. Sophias and K. Lourou Str
Athens (611)
Greece

CASE REPORTS

PREGNANCY IN A CASE OF NELSON'S SYNDROME

Sara Leiba, Haima Kaufman, Gideon Winkelsberg and Charles M. Bahary

From the Department of Endocrinology, the Department of Obstetrics and Gynecology and the Endocrine Laboratory, Beilinson Medical Center, Tel Aviv University Medical School, Petah Tikva, Israel

Abstract A woman suffering from Cushing's disease from the age of 17 who had been treated consecutively with pituitary irradiation, bilateral partial adrenalectomy and o,p-DDD (Mitotane USP) presented the clinical picture of Nelson's syndrome (hypoadrenalism with secondary hypersecretion of ACTH and MSH) at the age of 37. Under substitution therapy with corticoids she became pregnant for the first time at the age of 38. The course of the pregnancy was normal and at term she was delivered of a normal child by Cesarean section. The maternal-fetal relationship, the increased risk of pituitary infarction during pregnancy and the possible teratogenic effect of chemotherapy in such cases are discussed.

In most cases of Cushing's syndrome that have undergone chemical or surgical adrenalectomy there is permanent hypersecretion of ACTH and MSH. In a considerable number of these cases the hypersecretion is associated with hyperplasia of the pituitary gland, adenoma formation and secondary enlargement of the sella turcica (Nelson's syndrome). A woman who becomes pregnant under such conditions poses a number of problems in relation both to herself and her child. We recently had under our care a patient in whom Nelson's syndrome appeared at the age of 32 following treatment for Cushing's disease by bilateral partial adrenalectomy and pituitary irradiation as well as o,p-DDD (Mitotane USP). She became pregnant at the age of 38. The following is the case report of this patient.

CASE REPORT

The patient had suffered from Cushing's syndrome from the age of 17 years. She was given irradiation to the pituitary to a total dose of 30,000 r over a period of 6 years. At the age of 21 symptoms recurred and she underwent bilateral partial adrenalectomy. Three years later there was another recurrence and chemotherapy was instituted with o,p-DDD in a dose of 5 g daily for a total dose of 500 g. At the age of 32 the patient presented with hypoadrenalism and required substitution therapy with corticoids. Periodic ex-

amination of the sella turcica, visual fields and routine analyses were normal with the exception of the low levels of adrenal steroids and the high level of blood ACTH (350 pg/ml) (Table I).

The patient's menarche occurred at the age of 13 and she menstruated normally until the first symptoms of Cushing's disease developed. She was amenorrhoeic throughout the period of excessive adrenal activity but began to menstruate again once the level of blood corticoids had returned to normal. Beginning in 1968, under substitution therapy with corticoids, her menstrual cycles were relatively normal.

In 1975 the patient became pregnant and was very anxious to have the baby. It was decided to allow her to continue the pregnancy, taking particular care to carry out visual field and gynecological examinations as well as endocrine tests at frequent intervals. There was a moderate increase in the levels of urinary 17 OHCS, 11 OHCS and estradiol and in the levels of blood 11 OHCS but a sharp rise in plasma ACTH levels, the maximum being found one week before delivery (Table I). The patient became more pigmented but had no headaches and the visual fields remained normal.

In the 36th week of gestation the patient appeared with regular uterine contractions and a Cesarean section was performed. Two hours prior to operation and during the operation she received 100 mg hydrocortisone which was gradually reduced following delivery. The infant, a female weighing 600 g, was found to be in good health and was not pigmented. The post-operative course was uneventful.

DISCUSSION

This case of hypoadrenalism and secondary hypersecretion of ACTH had undergone treatment with both irradiation to the pituitary and a cytostatic drug. Her relatively advanced age, when there is a greater likelihood of chromosomal aberration, also contributed to the factor of risk. Pregnancy under these conditions raises a number of questions worthy of consideration: 1) the function of the fetal pituitary axis when the mother is receiving substitution therapy with corticoids and has a permanent hypersecretion of ACTH; 2) the increased danger of infarction of a hyperfunctioning pituitary gland dur-

Table I

Date	Urinary						Blood						Observations
	Vol	17KS	17OHC	11OHC	Total Estro- gens	Estrol	ACTH	11OHC	Estra diol	Proges terone	Testos terone	Aldos terone	
1973													
30 XI	450	2.5	6.1										Treatment hydrocortisone 70 mg/day
1975													
1 III							350						
9 III							315						
9 XI	600	1.2	5.8	33	16		400	6.4	600	23	25	7	Last menstruation
24 XI	1 150	1.9	6.6		65.5			6.9					20 VIII 75
23 XII	500	2.2	4.7	76	267	7.4	800	4.6		35	78.7	4.5	
1976													
1 II	600	1.3	5			5	>1500					8	Treatment meticorten 5-7.5 mg/day
23 III	560	2.8	5.7			6.4		9.4					fluonnel 0.1-0.2 mg/day
6 IV						10.7							Cesarean section
28 IV	700	3.2	7.4	196		17.7		13.4					25 V 75
15 V						16.5							
III V							>2160	11.7					
10 XI	700	2	2.8				1 000	1.8					Treatment meticorten 5-7.5 mg/day
7 XII	600	3.2	4.1	22.6			600	2.9					fluonnel 0.1-0.2 mg/day

Normal levels

Urinary

17KS 8-14 mg/24 h
 17OHC 4-16 mg/24 h
 11OHC 50-140 µg/24 h
 Total estrogens 15-35 µg (midcycle peak)

Estrol in pregnancy

16 weeks 2 mg/24 h
 20 weeks 5-8 mg/24 h
 27 weeks 10 mg/24 h

Blood

ACTH 10-80 pg/ml (RIA—a kit supplied by Radiochemical Ltd Amersham England)
 11OHC 11-23 µg/24 h
 17 beta estradiol (in pregnancy) 250 pg (greater than midcycle peak)
 Progesterone (in pregnancy) 17 mg/ml (greater than luteal phase level)
 Testosterone 20-60 ng/100 ml
 Aldosterone 5-16 ng/100 ml

ing pregnancy 3) the possible cytotoxic and teratogenic effect of the o.p. DDD even though it was administered a number of years before the pregnancy

1) The literature contains data (2, 4, 6, 9) indicating that pregnancy may compensate maternal cortical adrenal insufficiency through transfer to the mother of part of the corticoids secreted by the fetal adrenals. Glucocorticoids are able to cross the placental barrier in both directions influencing the function of both the maternal and fetal pituitary-adrenal axis. ACTH in contrast cannot cross this barrier (1, 5, 7, 10) and the maternal ACTH level has no influence on the fetal pituitary-adrenal function. It is concluded that hypersecretion of ACTH should not be considered a contraindication for pregnancy.

2) It is known that the pituitary gland enlarges appreciably during normal pregnancy and regresses rapidly but incompletely after delivery. There have been several reports of rapid tumor growth during pregnancy requiring emergency treatment (3). For this reason some clinicians recommend irradiation of the pituitary prior to the administration of fertility drugs in patients presenting with infertility and pituitary enlargement.

In our patient the sella turcica was not enlarged but there was evidence that the pituitary was hyperactive despite the considerable dose of radiation she had received in the past. The level of ACTH, which was significantly elevated at the beginning of pregnancy, subsequently rose considerably until the last month before delivery. Fortunately the patient felt well, had no headaches, and

sal fields remained normal throughout the pregnancy and after delivery.

DDDD is a cytotoxic drug specific for tumoral cells. Since the adrenals and gonads are of the same embryological origin, it is possible that the drug might have a teratogenic effect on the germinal cells, thus affecting future offspring. Luton et al (8) reported two patients who gave birth to normal infants after having received DDDD one year after conception and the other during therapy. These cases, together with ours, strongly suggest that treatment with DDDD, particularly when it has been instituted long before conception, is not a contraindication to pregnancy.

The factor which finally determined our decision to allow this patient to continue with her pregnancy was her strong desire to have a baby. It must be stressed, however, that in all similar cases the risk of pituitary infarction remains the most important risk. Thus each such case must be assessed carefully before the patient is allowed to continue a pregnancy.

REFERENCES

1. Allen J P, Cook D M, Kendall J W et al. Maternal fetal ACTH relationship in man. *J Clin Endocrinol* 37: 730, 1973.
2. Bayard F, Ances I G et al. The transplacental passage of prednisone and prednisolone in pregnancy near term. *J Pediatr* 81: 936, 1972.
3. Child D F, Gordon H, Mashiter M et al. Pregnancy, prolactin and pituitary tumors. *Br Med J* 4: 87, 1975.
4. Jackson T, Rauschecker H F J & Piasecki G Y. Quantitative relations of fetal and maternal pituitary adrenal systems. *J Clin Invest* 57: 3154, 1973.
5. Johannisson E. The foetal adrenal cortex in the human. *Acta Endocrin (Kbn) Suppl* 130: 7, 1968.
6. Jorgensen P I, Sele V, Buus O et al. Detailed hormonal studies during and after pregnancy in a previously hypophysectomized patient. *Acta Endocrin (Kbn)* 73: 117, 1973.
7. Kiyoshi A, Yoshimori K & Shioichi O. The effect of adrenocorticotrophic hormone and dexamethasone administered to the fetus in utero upon maternal and fetal estrogens. *Am J Obst Gynec* 113: 316, 1972.
8. Luton J P, Remy J M, Valke J C et al. Guérison ou rémission de la maladie de Cushing par usage thérapeutique prolongée de DDDD. *Ann Endocr (Paris)* 34: 351, 1973.
9. Milkovic K, Paunovic Y, Kniewald Z et al. Maintenance of the plasma corticosterone concentration of adrenalectomized rat by the fetal adrenal glands. *Endocrinology* 93: 115, 1973.
10. Simmer H H, Tulchinsky D, Gold E et al. On the regulation of estrogen production by cortisol and ACTH in human pregnancy at term. *Am J Obstet Gynecol* 119: 283, 1974.

Submitted for publication April 28, 1977

Sara Leiba
Department of Endocrinology
Tel Aviv University Medical School
The Beilinson Medical Center
Tel Aviv
Israel

Table I

Date	Urinary						Blood						Observations
	Vol	17KS	17OHS	11OHS	Total estro gens	Estron	ACTH	11OHS	Estra diol	Proges terone	Testos terone	Aldos terone	
1973													
30 XI	450	2.5	6.1										Treatment hydrocortisone 20 mg/day
1975													
1 III							350						
9 III							315						
9 XI	600	1.2	5.8	33	16		400	6.4	600	23	25	7	Last menstruation
24 XI	1 150	1.9	6.6		65.5			6.9					20 VIII 75
23 XII	500	2.2	4.7	76	267	2.4	800	4.6		35	28.7	4.5	
1976													
1 II	600	1.3	5			5	>1 500					8	Treatment meticotien 4-7.5 mg/day
23 III	560	2.8	5.7			6.4		9.4					fluonnel 0.1-0.2 mg/day
6 IV						10.7							
28 IV	700	3.2	7.4	196		17.7		13.4					Cesarean section
15 V						16.5							75 V 75
18 V							>2 160	11.2					
10 XI	700	2	2.8				1 000	1.8					Treatment meticotien 5-7.5 mg/day
7 XII	600	3.2	4.1	27.6			600	2.9					fluonnel 0.1-0.2 mg/day

Normal levels

Urinary
17KS 8-14 mg/24 h
17OHS 4-16 mg/24 h
11OHS 50-150 µg/24 h
Total estrogens
15-35 µg (midcycle peak)

Estron in pregnancy
16 weeks 2 mg/24 h
20 weeks 5-8 mg/24 h
27 weeks 10 mg/24 h

Blood

ACTH 30-80 pg/ml (RIA—a kit supplied by Radiochemical Ltd Amersham England)
11OHS 11-23 µg/24 h
17 beta estradiol (in pregnancy) 750 pg (greater than midcycle peak)
Progesterone (in pregnancy) 17 mg/ml (greater than luteal phase level)
Testosterone 20-60 ng/100 ml
Aldosterone 5-16 ng/100 ml

ing pregnancy 3) the possible cytotoxic and teratogenic effect of the o.p. DDD even though it was administered a number of years before the pregnancy.

1) The literature contains data (2, 4, 6, 9) indicating that pregnancy may compensate maternal cortical adrenal insufficiency through transfer to the mother of part of the corticoids secreted by the fetal adrenals. Glucocorticoids are able to cross the placental barrier in both directions, influencing the function of both the maternal and fetal pituitary-adrenal axis. ACTH, in contrast, cannot cross this barrier (1, 5, 7, 10) and the maternal ACTH level has no influence on the fetal pituitary-adrenal function. It is concluded that hypersecretion of ACTH should not be considered a contraindication for pregnancy.

2) It is known that the pituitary gland enlarges appreciably during normal pregnancy and regresses rapidly but incompletely after delivery. There have been several reports of rapid tumor growth during pregnancy requiring emergency treatment (3). For this reason some clinicians recommend irradiation of the pituitary prior to the administration of fertility drugs in patients presenting with infertility and pituitary enlargement.

In our patient the sella turcica was not enlarged but there was evidence that the pituitary was hyperactive despite the considerable dose of radiation she had received in the past. The level of ACTH, which was significantly elevated at the beginning of pregnancy, subsequently rose considerably until the last month before delivery. Fortunately the patient felt well, had no headaches, and the

THE LANDRY-GUILLAIN-BARRE SYNDROME AND PREGNANCY

Greger Ahlberg and Gosta Ahlmark

From the Department of Medicine Falu Hospital Falun S eden

The Landry-Guillain-Barre syndrome is considered rare in connection with pregnancy. In mild cases the course of the pregnancy is unaffected. In severe cases with respiratory depression and bulbar symptoms, especially during late pregnancy, the syndrome entails an increased risk to both mother and foetus. Therapeutic abortion or cesarean section are not considered to be indicated. A pregnant woman developed the disease during the final trimester and gave birth to premature twins during respiratory treatment. The mother and the infants survived.

LGBS during pregnancy is rare and only a few cases have been described in the literature. Most of the mothers have had the disease in relatively mild form. This communication reports one of the few cases with respiratory depression in which the mother and offspring survived. It is also the first twin pregnancy reported in connection with LGBS.

CASE REPORT

The patient was a 38-year-old married primigravida woman who on 5th August, in the 32nd week of pregnancy, developed paraesthesiae in her hands and feet without preceding infection. During the following ten days she experienced progressive muscle weakness in her arms and legs and also headache. On 14th August she developed right-sided facial paresis and came to the hospital. The course of the pregnancy had been completely normal but a twin pregnancy was suspected.

The patient was mentally alert and showed signs consistent with the 3rd week of pregnancy. The foetal sounds were normal. Blood pressure was 130/80 mmHg and the ECG was normal. A neurological investigation showed right-sided facial paresis but the other cranial nerves were intact. Incomplete paresis was present in all extremities and the patient was unable to stand up. The peripheral reflexes were weak and no sensory symptoms could be detected. Babinski's reflex was absent bilaterally.

On 16th August further progression had occurred and the patient now had difficulty in talking and swallowing, incipient double vision and also left-sided facial paresis. On the following day she had difficulty in controlling her micturition and was treated with catheter à demeure. The pharyngoparalysis and paresis of the extremities became total and respiration deteriorated.

Laboratory routine tests were normal. Lumbar puncture on 15th August showed free Queckenstedt bilaterally and clear CSF without cells and the protein concentration was 570 mg/l. On 17th August the CSF protein concentration was 640 mg/l and no cells were found. The preliminary diagnosis of the Landry-Guillain-Barre syndrome was considered confirmed.

On 20th August respiratory failure occurred and respirator treatment was started. A lung X-ray showed basal atelectasis on the left side. Tracheotomy was performed. During preparations for this procedure the water broke. Labour started nine hours after the patient had been

Landry (15) described in 1859 a neurological disorder with ascending paralysis which was further defined by the specific cerebrospinal fluid (CSF) findings of Guillain Barre & Strohl in 1916 (11). This disease—the Landry-Guillain-Barre syndrome (LGBS) or acute polyradiculitis—is a demyelinating neuropathy of unknown aetiology. The disease affects people of all ages and is somewhat more common in men than in women. Clinically demonstrable infection precedes the disease in more than 50% of the cases (16) and a postinfectious allergic immunological reaction has been suggested as the aetiological background. Patho-anatomically there is segmental demyelination of peripheral nerves and in severe cases perivascular inflammatory infiltration of the nerve roots occurs.

Typical LGBS is characterized by symmetric motor symptoms with ascending paresis which may affect both spinal and cranial nerves. This paresis with impaired tendon reflexes may progress within a period of two weeks and lead to bulbar symptoms and respiratory depression. Sensory symptoms in the form of paraesthesiae and muscle pain are also usually present. The CSF findings are characterized by increased protein without a corresponding increase in cells—albuminocytological dissociation. A slight increase in mononuclear cells may occur. The patient usually recovers spontaneously within a few months but residual symptoms occur and the mortality is not negligible.

placed in the respirator. Delivery was achieved by suction. Both infants who were monozygotic boys had Apgar scores of 1 but their status became normal within six hours.

The mother's condition was unchanged post partum but she was treated with penicillin because aspiration was suspected. Lactation was suppressed by hormone treatment.

During the following three days the patient's condition deteriorated to generalized total paralysis. She also had pains in all her extremities and in the muscles of her back. Her neurological status remained largely unchanged for the next three weeks apart from a subjective impairment of hearing which lasted a few days.

On 10th September the patient began to experience paraesthesiae in her hands and a few days later she was able to move her head slightly. The paraesthesiae then regressed very slowly and breathing exercises could be started. It was not until after 61 days (20th October) that respirator treatment could be stopped.

During this period the patient was treated with various antibiotics owing to infection in the tracheostoma and also pneumonia. She was given intensive physiotherapy.

On 20th August CSF protein was 900 mg/l and on 27th August it was 4070 mg/l (CSF electrophoresis showed massive barrier damage with a serum like pattern). Virus cultures and titrations were negative.

The patient was treated for a further four months at the Rehabilitation Department and was then able to leave hospital with only slight weakness of her extremities and vestigial bilateral facial paresis. Both babies were completely healthy when they left hospital.

DISCUSSION

Only 28 cases of the Landry-Guillain-Barre syndrome during pregnancy have been reported and the diagnosis is uncertain in some of these (2, 3, 6-9, 12, 14, 16, 18-21, 24-29). Several authors have claimed that the syndrome is very rare during pregnancy and it has been suggested that pregnancy per se protects the woman from LGBS (1, 2, 17, 30). Ravn (26) after reviewing a large number of cases concluded however that the syndrome was not exceptionally uncommon in pregnant compared to non pregnant women. Thus pregnancy does not seem to prevent the syndrome but nor does it influence the severity of the disease (19).

Osler & Sidell (20) suggested in 1960 a number of diagnostic criteria for LGBS. The case described here fulfils most of these criteria and the LGBS diagnosis may therefore be considered certain. Similar symptoms with polyneuropathy or muscle weakness may occur during pregnancy due to nutritional deficiencies particularly vitamin B deficiency associated with hyperemesis gravidarum (17). In LGBS the facial nerve is the most affected

cranial nerve and facial paresis during may be the only finding in very mild form of LGBS (4).

In large series of patients with LGBS the mortality has been between 10 and 70% increasing with age. The prognosis is considerably poorer with bulbar symptoms and respiratory depression present. It has been possible however to reduce this high mortality to about half (13, 36) by introduction of respirator treatment and improved antibiotic therapy.

Of the 28 pregnant women with LGBS reported in the literature three died (11%) all post partum. These three women were given respirator treatment at partus and two died later from infections and from haemorrhage from a tracheoarterial fistula (20, 27).

The onset of LGBS in the previously described pregnant women was not correlated to any particular point of time during the pregnancy (39). The course has been more serious when the onset occurred during the latter part of pregnancy and respiratory failure has occurred in many of these cases. In our patient the onset occurred in the 3rd week of pregnancy without being preceded by a definite infection. The clinical course was unusually prolonged and severe. Muscle weakness in the extremities and face was still evident six months after the onset. In an unselected series of patients, residual symptoms occurred in 5-10% (33). After function may however continue to improve for up to three years (22). According to the literature cranial nerves may be engaged except the olfactory and vestibulocochlear nerves (36). Our patient complained of impairment of hearing but audiometry could not be carried out and it was therefore not possible to verify objectively that the cochlear nerve was involved. The concentration of protein in the CSF was high (4070 mg/l) but this was thought not to be correlated to the severity of the disease (14, 26).

Early spontaneous abortion occurred in one of the 28 women suggesting that the frequency of abortion does not increase in connection with LGBS. In cases in which onset of the disease occurred during the first or second trimester the pregnancy proceeded without complications in these cases and partus was normal. When the onset occurred during the third trimester however there is an increased risk of premature labour.

It is well known that serious neurological

es do not generally depress the uterine contractions and LGBS does not seem to be an exception to this. The twin delivery took an obstetrically normal course in this case but suction had to be used during the expulsion phase. Assisted extraction has been necessary in most cases in which paresis of abdominal muscles has been present. Cesarean section has been used in three cases and two mothers both of whom were treated in a respirator died post partum whereas the third woman who had a mild form of LGBS at the time of partus survived.

Since only a few cases of LGBS during the early part of pregnancy have been described in the literature it is not possible to draw certain conclusions as to whether or not the neurological disease is hereditary. No case of congenital malformations has been reported, however.

Of the 79 pregnancies (involving 30 foetuses/babies) complicated by LGBS that have been reported so far, information is lacking for three in which spontaneous abortion occurred in one case and one therapeutic abortion was performed in the fourth week. Twenty-two babies survived and 17 of these were premature. Three babies died shortly after partus from complications not definitely related to LGBS (7/19/28). The infant survival was thus at least 88%.

The mothers of the five surviving premature babies all fell ill during the final trimester. The clinical course was severe and necessitated respiratory treatment in all cases and two of these babies died post partum. None of the surviving infants has had symptoms of neurological disease. LGBS has thus not been transmitted from mother to offspring.

In view of the good prognosis for the baby most authors consider that therapeutic abortion is not indicated. Our twin birth supports this view.

TREATMENT

Treatment of patients with acute polyradiculitis is symptomatic and in severe cases is aimed above all at maintaining adequate ventilation and preventing infection. The prognosis in patients with respiratory failure has also improved greatly since respirator treatment came into general use (26). Six out of 29 pregnant women with LGBS have required respirator treatment. Whether the need for respiratory treatment is greater in pregnant than in non-pregnant women is difficult to say. Most

published series of patients with LGBS are selected with a high proportion of severe cases.

Steroid therapy was previously considered beneficial in certain cases (1/12/14) and Notter (19) used large doses of steroids without negative effects of the foetus. More recent experience has not confirmed the effect of corticosteroids in LGBS, however, irrespective of whether the patient is pregnant or not (10/13/26). It is important that physiotherapy is given throughout the period of illness (5).

CONCLUSIONS

Only a few cases of LGBS in connection with pregnancy have been reported up to now. In mild cases pregnancy does not seem to exacerbate the disease and the pregnancy often takes a completely normal course. The risk of malformations, spontaneous abortion or foetal death seems not to increase. In severe cases with complicating respiratory failure and onset during the last trimester, however, the risk to the mother and incidence of premature birth seems to increase greatly. Most authors are agreed that abortion is not indicated either from the mother's or the baby's point of view. Nor is there a stronger indication for cesarean section when the mother has LGBS. Treatment is symptomatic only and there is no evidence that specific therapy such as corticosteroids is beneficial.

REFERENCES

1. Berlacher F J & Abington P B. ACTH and cortisone in Guillain-Barre syndrome. *Ann Med Intern* 48: 1106 1958.
2. Batson J & Golden M. Guillain-Barre syndrome in pregnancy. *Obstet Gynecol* 15: 311 1960.
3. Blemond A. Über die Meningo-Radiculo-Neuritis (Guillain-Barre) und die Meningomyelo-Encephalitis betrachtet als Krankheitsform bei denen das pathologische Agens primär im Meningealraum angreift. *Dtsch Z Nervenheilk* 143: 172 1957.
4. Charous D I & Saxe P. The Landry-Guillain-Barre syndrome. *N Engl J Med* 277: 1334 1967.
5. Conomy J & Prader J. Guillain-Barre syndrome: The physical therapist and patient care. *Phys Therapy* 51: 517 1971.
6. Elstein M, Lezz J J, Murphy M, Park D M & Sutcliffe M I. Guillain-Barre syndrome in pregnancy. *Anaesthesia* 27: 216 1971.
7. Feldman S, Lardos J & Halpern L. Polydema in the Guillain-Barre syndrome. *Arch Neurol Psychiatry* 73: 172 1973.
8. Gardner W H, Butler D K & Whitten C. Increased intracranial pressure caused by increased

- protein content in the cerebrospinal fluid *N Engl J Med* 250 932 1954
- 9 Gendre Tourmilhac Mustier Lafarge A propos d'un cas de syndrome de Guillain-Barre au cours de la grossesse *Gaz Med France* 70 2405 1963
 - 10 Godall J A D Kosmidis J C & Geddes A M Effect of corticosteroids on course of Guillain-Barre syndrome *Lancet* 1 524 1974
 - 11 Guillain G Barre J A & Strohl A Sur un syndrome de radiculonévrite avec hyperalbuminose du liquide céphalo-rachidien sans réaction cellulaire. Remarques sur les caractères cliniques et graphiques des reflexes tendineux *Bull Soc Med Hop Paris* 40 1462 1916
 - 12 Heller G & DeJong M Treatment of the Guillain-Barre syndrome Use of the corticotropin and glucocorticoids *Arch Neurol* 8 179 1963
 - 13 Hughes R A Perkin G B Stern G M Davis J N & Thomas P K Multicentre trial of prednisolone in the Guillain-Barre syndrome *Br Med J* 4 461 1975
 - 14 Kalstone B M Pearce III F Guillain-Barre syndrome in a pregnant woman *Am Pract* 10 674 1959
 - 15 Landry O Note sur la paralysie ascendante aigue *Gaz Hebd Sci Med Bordeaux* 6 472 1859
 - 16 Marsel J & Woltman H Neuritis of pregnancy without vomiting *JAMA* 103 1930 1934
 - 17 McGoogan L & Omaha N Severe polyneuritis due to vitamin B deficiency in pregnancy *Am J Obstet Gynecol* 43 757 1947
 - 18 Muller M Polyradiculoneuritis Guillain-Barre and pregnancy *Cs Gynecologie* 40 674 1975
 - 19 Notter A & Gaja R Syndrome de Guillain-Barré et grossesse *Lyon Med* 223 156 1970
 - 20 Osler L & Sidell A The Guillain-Barre syndrome The need for exact diagnostic criteria *N Engl J Med* 262 964 1960
 - 21 Pavlovic J Varga I & Tomic M Respiratory failure of idiopathic polyradiculoneuropathy in advanced pregnancy *Serb Med Arch* 93 471 1965
 - 22 Pieterman A Daly M Dion F & Kerh M Infectious neuritis (Guillain-Barré syndrome) children *Neurol* 9 533 1959
 - 23 Petlund C F Polyradiculitis Guillain-Barré et l'arsmateriale (Review of 10 years LGBS cases) *N Lægeforen* 82 1139 1967
 - 24 Provvidenza G Reverben L & Flona M Il syndrome di Guillain-Barre in gravidanza *Rass G Gera* 64 175 1965
 - 25 Radman M Guillain-Barre disease complicated pregnancy *Maryland Med J* 16 54 1967
 - 26 Ravn H The Landry-Guillain Barré syndrome *Acta Neurol Scand Suppl* 30 1967
 - 27 Rudolph J Norris F Garvey M & Fredenck J The Landry-Guillain-Barre syndrome in pregnancy a review *Obstet Gynecol* 26 265 1965
 - 28 Spire M Polyneurite gravidique sans vomissement incoercibles *Bull Soc Obstet Gynecol* 2 500 1913
 - 29 Sudo N & Weingold A Obstetric aspects of the Guillain-Barre syndrome *Obstet Gynecol* 43 1975
 - 30 Zfass H S Zfass I S Troland C E & Mack R Guillain-Barre syndrome report of a case in pregnancy and a review of the literature *Virginia M Mon* 81 77 1954

Submitted for publication May 19 1977

Greger Ahlberg
Department of Medicine
Falun Hospital
Falun Sweden

RECURRENT HYDATIDIFORM MOLE

Report of a Case with Five Recurrences

Eva Patek and Per Johnson

*From the Department of Obstetrics and Gynaecology
Huddinge sjukhus Huddinge Sweden*

Abstract A report on a patient having five consecutive molar pregnancies is presented. None of the pregnancies was associated with a fetus and all five hydatidiform moles were histologically benign. The treatment of recurrent moles is discussed and the literature concerning this problem is reviewed.

The occurrence of a hydatidiform mole is rare in industrialized countries where it is reported to develop in the proportion of 1/2 500 pregnancies (5-7). Degenerations of pregnancy are more frequent in South East Asia and Latin America probably due to protein deficiency where its incidence is calculated to be 1/82 (8-9-11-12).

Recurrent hydatidiform mole is an extremely unusual condition with a reported incidence in the range of 1 in 75 molar pregnancies (13). The recurrent mole appears in two main patterns, i.e. in a person with normal pregnancies and the subsequent repetition of molar gestations (14).

The purpose of this paper is to present a case report of a patient with five hydatidiform moles to discuss the treatment and to review the literature concerning this subject.

CASE REPORT

The patient, a 33-year-old gravida 5 para 0 was first seen at the Huddinge University Hospital in October 1976.

1st pregnancy
The first pregnancy occurred at the age of 24. She was treated for threatening abortion in the first trimester of pregnancy. The patient started to bleed again in the 13th week and subsequently the uterus failed to grow. In the 17th week it reached the umbilicus and after slight delay the patient passed a bunch of grape-like vesicles. Evacuation was performed the same day and histological examination showed a benign mole. HCG was within normal limits during the pregnancy and repeatedly negative. Arteriography of the pelvis was normal.

2nd pregnancy

The patient was advised against pregnancy until a year had passed. Her second pregnancy was uneventful except for intermittent vaginal haemorrhage. The uterus was developing normally for the time of gestation and HCG was also normal. During the last 6 weeks before admission, however, the uterus failed to enlarge, fetal movements were never felt and at the 30th week of gestation the uterus reached the umbilicus. X-ray examination of the pelvis revealed no fetus and on the same day the patient spontaneously passed a hydatidiform mole which was histologically benign. Arteriography 7 weeks later was normal and HCG negative.

3rd pregnancy

The third pregnancy occurred 2 years later. The uterus failed to enlarge after the 15th week; the patient had vaginal bleeding and suffered from hyperemesis. However, HCG values were within normal limits. After the administration of ethinyl estradiol in the 30th week a hydatidiform mole was delivered and evacuation was performed. The mole was histologically benign and an arteriography was negative as were several HCG tests.

4th pregnancy

The fourth pregnancy took place 1½ years later and was uneventful until the 18th week of gestation when the growth of the uterus ceased, although HCG was normal. Ultrasound in the 23rd week diagnosed a hydatidiform mole as did X-ray of the pelvis. After the administration of ethinyl estradiol and an intravenous oxytocin infusion a mole was passed and the uterus was evacuated. The mole proved to be benign histologically and HCG was repeatedly negative. A chromosomal analysis of the patient was now considered after the 4th consecutive mole but unfortunately not undertaken. An X-ray of the chest was negative.

5th pregnancy

In her fifth pregnancy the patient had her last menstrual period on 1/7 1976. She was complaining of headache and hyperemesis and was suffering from slight intermittent vaginal bleeding. Repeated vaginal examination disclosed that the uterus normally enlarged for the time of gestation until it reached the umbilicus when growth ceased. The

uterus subsequently rather decreased in size. HCG was 185 000 IU/litre in the 14th week and 238 000 IU/litre in the 16th week. FHS were never heard and the patient had never felt any movements. In the 23rd week an ultrasound examination disclosed a picture typical of a hydatidiform mole. Labour was induced with oxytocin intravenous infusion and the uterine cavity was explored and evacuated by vacuum aspiration of typical grape-like moles. Histological examination of the specimen displayed a benign mole.

The family history of the patient showed that her sister had two children but had also had a hydatidiform mole. The patient and her husband have now decided against further pregnancies and in the belief of an increasing risk of development into a choriocarcinoma after each successive mole sterilization will be performed as an elective hysterectomy. The family has applied for an adoptive child.

DISCUSSION

The repeat hydatidiform mole has always been considered to be an extreme rarity. Most such moles reported in the literature have only recurred twice (barely 60 cases reported with two recurrences) although as many as 18 repetitions in one and the same patient were reported by Essen Møller in 1912 (5).

So far only 43 cases including that of our own of a hydatidiform mole recurring 3 or more times have been reported (10-12). However many of the cases remain doubtful as some instances of moles recurring more than six times were based only on the history offered by the patient and were not confirmed by any histological evidence. Moreover some investigations were probably dealing with the growth of a persisting mole rather than with a new pregnancy. In the present study with 5 recurrent hydatidiform moles in the span of 9 years there is evidence of the absence of molar tissue between each pregnancy.

The reported incidence of recurrent hydatidiform mole varies between 1 in 75 molar pregnancies (1.3% Europe) (13) and 1/23 or 4.37% in another series (Mexico) (12). Incidences of 1/50 have been recorded by two independent groups corresponding to a frequency of 2% (11). This low incidence is partially explained by the fact that many patients are still treated with hysterectomy without previous curettage as proposed by Acosta Sison (1) and partly because a molar pregnancy is most common in the 5th decade when a woman's fertility is low. Thus it is not surprising that recurrent hydatidiform mole is uncommon since less than

half of the patients with molar degeneration become pregnant again (4-7).

Close observation of repeated molar pregnancy for possible malignancy has been advocated by most authors (1, 4, 6-9). There is a suggestion in the literature that the trophoblastic malignancy might be higher in recurrent than in isolated moles (1, 7-9). The incidence of choriocarcinoma has been calculated to be 7.9% after each mole (1). The same author has calculated an incidence of choriocarcinoma/patient of 23% in repeat occurrences. This high incidence prompted several investigators to use cytotoxic agents as a prophylaxis against malignant trophoblastic disease in case of recurrent benign moles (8, 9). However there are indications that these figures might be too high. The calculations were based on selected cases, some with a favourable outcome were lost to follow up and thus omitted.

Brandes & Peretz (2) have suggested that the term 'habitual mole' should be used after 3 moles as 'habitual abortion' is used after 3 abortions. They postulated that this might be an entirely different entity, i.e. no choriocarcinoma malignancy hitherto been reported in patients where hydatidiform mole has recurred more than twice. They suggested that the rate of choriocarcinoma might decrease even to extinction in cases of recurrence on 3 or more occasions. It is also striking that in these cases with habitual mole a normal pregnancy interspersed in the history of moles is very rare.

A series of 43 known cases of habitual mole, some of them even doubtful, is too small to draw any conclusions as to their possible malignant outcome. It is therefore advisable to treat each individual case on its own merits. Close surveillance is necessary in all cases but hysterectomy can be recommended after a certain arbitrary number of moles unless other criteria warrant it.

REFERENCES

1. Acosta Sison H. Indications for immediate hysterectomy without curettage in cases of hydatidiform mole. *Am J Obstet Gynecol* 81: 715-19.
2. Brandes J & Peretz A. Recurrent hydatidiform mole. Report of a case. *Obstet Gynecol* 35: 398-19.
3. Chesley L C, Cosgrove S A & Preece Hydatidiform mole with special reference to recurrence and associated eclampsia. *Am J Obstet Gynecol* 57: 311-1946.

1. Diamond B A & Spellacy W N Recurrent hydatidiform mole Report of a case of four recurrences and a review of the literature Wisc Med J 67 16 1968
2. Eisen-Moller E Studien über die Blasenmole (ed J F Bergmann) Wiesbaden 1912
3. Goldman J A & Eckerling B Recurrent hydatidiform mol Review of the literature and report of a case Israel Med J XX 316 1961
4. Hertz A T & Sheldon W H Hydatidiform mole Apthohistoclinical correlation of 200 cases Am J Obstet Gynecol 53 1 1947
5. Hsu C T, Cheng T Y, Chiu W H, Yang C-C, Lu C H, Changcheng C H, Tung P H & Chen C-C. Some aspects of trophoblastic diseases peculiar of Taiwan. Am J Obstet Gynecol 90 308 1964
6. Hsu C Y, Lau C H, Changchein C L & Changchein B H Repeat hydatidiform moles Report of seven cases Am J Obstet Gynecol 87 543 1963
7. Kronfol N M, Ilja F A & Hay S N Recurrent hydatidiform mole A report of five cases with review of the literature Leb Med J 27 507 1969
8. Matalon M & Modan B Epidemiologic aspects of hydatidiform mole in Israel Am J Obstet Gynecol 112 107 1972
9. Molina G V, Ramos J N & Dyran A A Mola de repetición Ginec Obstet Mex 37 III 1972
10. Morrison D L Recurrent hydatidiform mole A case report of 3 consecutive moles J Obstet Gynaecol Br Comm 72 749 1965
11. Wu F Y Recurrent hydatidiform mole A case report of nine consecutive molar pregnancies Obstet Gynecol 41 200 1973

Submitted for publication Feb 21 1977

Eva Patek
Huddinge Hospital
S-141 86 Huddinge
Sweden

GRANULAR CELL MYOBLASTOMA OF THE VULVA

REPORT OF 4 CASES

Ram Dgani Bernard Czernobilsky Richard Borenstein
and Moshe Lancet

From the Department of Obstetrics and Gynecology and the Department of Pathology, Kaplan Hospital, Rehovot, Israel. Affiliated to the Medical School of the Hebrew University and Hadassah, Jerusalem, Israel.

Granular cell myoblastoma is a rare tumor for which the vulvar area is one site of predilection. It occurs at any age as a firm, rounded nodule usually not exceeding 3 cm in diameter (10). The current belief is that this tumor may be derived from granular cells (4, 8). Only 49 cases of granular cell myoblastoma of the vulva have so far been reported (3).

CASE REPORTS

Case 1 The patient, a 67-year-old Jewish female, gravida 4 para 3, abortion 1, was seen on November 3, 1975 in the gynecologic clinic because of a lump on the genitalia noted for approximately 6 months. The lump had caused some itching but it was not tender. A firm, nontender 3 mm tumor was noted on the middle portion of the right labium majus. The tumor was freely movable.

The preoperative impression was that of a sebaceous cyst. The tumor was excised with a border of normal tissue. Upon section, it was seen to be firm, pale yellow, homogeneous and apparently not encapsulated. This was in accordance with the description of granular cell myoblastoma in the literature. The patient has been followed for 18 months without local recurrence or appearance of a similar tumor elsewhere.

Case 2 This 46-year-old Jewish female, gravida 4 para 3, abortion 1, was seen in December 1976 with the complaint of a slowly growing lump on the genitalia noted for approximately 1 year. The tumor, measuring 2 cm in diameter, was located on the anterior portion of the left labium majus. It was hard and not tender. The impression was that of a lipoma.

The lesion was excised with a 1 cm border of normal tissue. The tumor presented the same gross features of a granular cell myoblastoma.

No recurrence has been noted in a 6-months follow up. **Case 3** This 17-year-old single Jewish female presented herself in May 1976 for treatment of a recently noted lump on the genitalia. Physical examination revealed a firm nodule 7 cm in diameter involving the right

labium majus. Under local anesthesia wide excision of the nodule was performed. The tumor showed the macroscopic details of a granular cell myoblastoma. During the year since her operation no recurrence has been noted.

Case 4 A 35-year-old Jewish female, gravida 4 para 4, was seen in August 1971 because of vulvar lump that appeared one year before and has increased in size recently. A firm 1 cm × 1 cm nodule was noted on the left labium majus.

The tumor was excised with a border of normal tissue. The cut surface was grossly similar to those described above but it was whitish. There has been no evidence for recurrence during the ensuing 5 years.

Histologic examination of the 4 tumors revealed a similar picture in all of them. It consisted of large cells with a finely periodic acid-Schiff positive granular cytoplasm and small round centrally placed regular nuclei. The cells had poorly defined cytoplasmic borders. The lesions were present within the dermis and subcutaneous tissue and lacked encapsulation. The overlying squamous epithelium demonstrated some areas of irregular proliferation in cases 2 and 4. The tumors were all completely excised (Figs. 1-3).

COMMENT

Only about 7% of the granular cell myoblastoma have been located in the vulva. About 35% of these tumors have involved the tongue and the rest have been found in the skin and various other sites. Age incidence has varied from 6-70 years (2, 3).

On clinical examination a granular cell myoblastoma presents generally as a small, solitary, painless mass and may be similar to lipoma, fibroma, hidradenoma, papilloma, sebaceous cyst and Bartholin's gland tumor (2). If ulceration of the overlying skin occurs it may simulate carcinoma or venereal disease (3). Multiple tumors are reported in 3-16% of cases. This tumor may recur locally and



Fig 1 Vulvar tissue showing some irregular proliferation of the overlying squamous epithelium and diffuse infiltration of dermis and subcutaneous tissue by ill-defined cells with small round nuclei. Hematoxylin Eosin $\times 100$

rarely may be malignant (6-9). In such cases metastatic spread occurs in general by lymphatic channels and causes death regardless of therapy (5, 6).

Recent evidence has suggested that this tumor is of neural origin. Electron microscopy and histo-

chemical studies of the tumor revealed similarity to Schwann cells and it was felt that granular cell myoblastoma is a misnomer and the more appropriate name should rather be granular cell Schwannoma (4, 8). Previous reports of granular cell myoblastomas which were found within the

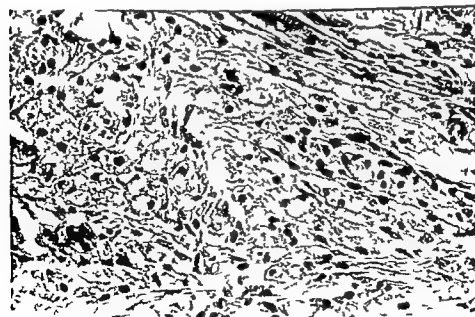


Fig 2 High magnification of granular cell myoblastoma showing typical granular cytoplasm and centrally placed nuclei of cells. Hematoxylin Eosin $\times 250$

head of peripheral nerve (1) and within the hypophyseal stalk (7) have supported a neural histiocytosis. The accompanying hyperplastic response of the overlying squamous epithelium may occasionally simulate a squamous cell carcinoma (4, 10).

The tumors are radio resistant usually not encapsulated and may recur locally. Therefore wide local excision was recommended as the treatment of choice (3). If a malignant granular cell myoblastoma is found radical vulvectomy with regional node dissection should be considered. Radiation therapy or chemotherapy has little or no value (1).

REFERENCES

1. Bunge H J Jr. An early granular cell myoblastoma confined within a small peripheral myelinated nerve. *Cancer* 6: 790 1953.
2. Birch H W & Sondag D R. Granular-cell myoblastoma of the vulva. *Obstet Gynecol* 18: 443 1961.
3. Coates J B & Hales J S. Granular cell myoblastoma of the vulva. *Obstet Gynecol* 41: 796 1973.
4. Fisher E R & Wechsler H. Granular cell

myoblastoma—a misnomer. Electron microscopic and histochemical evidence concerning its Schwann cell derivation and nature (granular cell Schwannoma). *Cancer* 15: 936 1962.

5. Gamboa L G. Malignant granular cell myoblastoma. *Arch Pathol* 60: 663 1955.
6. Gifford R R M & Birch H W. Granular cell myoblastoma of multicentric origin involving the vulva. A case report. *Am J Obstet Gynecol* 117: 184 1973.
7. Harland W A. Granular cell myoblastoma of the hypophyseal stalk. *Cancer* 6: 1134 1953.
8. Sobel H J, Marquet E & Schwarz R. Is Schwannoma related to granular cell myoblastoma. *Arch Pathol* 95: 396 1973.
9. Strong E W & McDevitt R W & Brasfield R D. Granular cell myoblastoma. *Cancer* 25: 415 1970.
10. Woodcock A S. *The Pathology of the Vulva*. Chap 4. Postgraduate Obstetrical and Gynecological Pathology. First Edition. Edited by H Fox F A Langley. 67. Pergamon Press New York 1973.

Submitted for publication August 8 1977

Moshe Lancet
Department of Obstetrics and Gynecology
Kaplan Hospital
Rehovot
Israel

ANNOUNCEMENTS

IUD and Biodegradable Delivery Systems (International Symposium Amsterdam Holland June 28-30 1979)

Thirty leading andrologists gynecologists and reproductive biologists have been invited to discuss

1 Kinetics & Application of IUD Copper IUD Steroid delivery systems for contraception Failure rate of IUD IUD and pregnancy IUD expulsion and related phenomena Post placental IUD insertion IUD and family planning IUD and inflammatory disease Future development of IUD and Management of intraperitoneal IUD

2 Physiology and Biochemistry Geometry of IUD and uterine lumen Physiology of uterine blood loss Physiological mechanisms of endometrial bleeding in the IUD Pathology of endometrial bleeding by IUD Factors affecting uterine blood loss Fibrinolytic agents Immobilization of spermatozoa Agglutination of spermatozoa

3 Biodegradable Delivery Systems Biodegradable polymers polyactides and polyglycolides Kinetics of release diffusion and erosion Biodegradable microcapsules and microspheres Use of bioerodible bioactive and biodegradable Toxicology and teratology of local delivery systems Homo and copolymers Biodegradable delivery systems Device design and system application A fibrinolytic agent release from IUD Multidimensional models for estimating & predicting IUD-related menorrhagia blood loss Decision system for selecting best IUD for individual acceptors Biodegradable polymers in improving postpartum IUD retention

This is a satellite symposium of PANCA to be held March in Caracas Venezuela Program Directors J van Os Haarlem Holland & E E Haefz Detroit US Symposium Office Miss K Sparyjaard P O Box 70 Oss Holland telex 50959

Xth World Congress of Fertility and Sterility

The International Federation of Fertility Societies will sponsor the Xth World Congress to be hosted by the Spanish Fertility Society at the National Palace of Expositions and Congresses in Madrid Spain September 20-26 1980 There will be sessions on Contributions to the Themes New Methods Workshops Luncheon Conferences Informal Sessions of Questions and Answers by experts Scientific Exhibits Motion Pictures etc There will be two days for Pre Congress Symposia and Post Graduate Courses followed by a program on the following themes

I Spermatogenesis (Induction and inhibition clinical and physiological aspects)

II Ovulation (Induction and inhibition clinical and physiological aspects)

III Psychosexual and social aspects of fertility (Psychosexual social)

IV Problems of gametes transportation (Female male)

V Immunology in reproduction (Female male)

VI Control of fertility (Female male)

VII Neuro-endocrinology of reproduction (Female male)

VIII Fertilization and implantation (Fertilization & implantation)

IX Environmental and iatrogenic aspects of reproduction (Environmental factors iatrogenic factors)

X Genetics in reproduction

Send inquiries to

Scientific Program Chairman Prof A Campos da P M D Av N S de Copacabana 664/607 Rio de Janeiro 20000-Brazil

President of the Congress Dr J Cortes Pardo M D Pro Bermeo 11 (Nursierra) Madrid 34 Spain

President of IFFS Dr P C Steptoe M D 61 Street West Oldham Lancs England

EFFECT OF CHLORTHIAZIDE TREATMENT ON RENIN-ALDOSTERONE SYSTEM DURING PREGNANCY

Risto Lammintausta Risto Erkkola and Matias Eronen

*From the Institute of Biomedicine Department of Pharmacology University of Turku
and the Department of Obstetrics and Gynecology Turku University
Central Hospital Turku Finland*

Abstract Plasma renin activity and urinary excretions of aldosterone sodium and potassium were studied before and during one weeks chlorthiazide treatment in eight women with a slight peripheral oedema in the 31st week of pregnancy. Plasma renin activity and excretion of aldosterone increased clearly on the first day of treatment but on the 7th day it was more than doubled in comparison with the level before the treatment. Diuresis and excretion of sodium increased promptly on the 1st day but did not differ from the level found before the treatment on the 7th day. The high reserves of renin and aldosterone secretion found in this study may be regarded important in ensuring blood pressure plasma volume and placental flow during pregnancy.

In the obstetrical literature contradictory statements exist on the usefulness of diuretic treatment during pregnancy. In some studies the prophylactic use of diuretics has been shown to decrease the incidence or severity of toxemia and also the neonatal mortality (8-13) whereas other investigators have found diuretics of no value or even harmful (3, 4). Diuretics are however in common use and there are areas in which more than half of the mothers receive this kind of therapy during their pregnancy (9).

The thiazide diuretics are known to activate the renin-aldosterone system (6-16). In one of our earlier studies (17) we demonstrated how the pregnancy also activates this system. In the 30th week of pregnancy the plasma renin activity (PRA) was increased approximately seven fold and aldosterone excretion in urine approximately 13 fold compared to non pregnant excretion. In this mind we planned and carried out our study firstly to observe the effect of diuretics on the renin-aldosterone system during pregnancy and secondly to detect whether the raised values of pregnancy could be increased any further.

SUBJECTS AND METHODS

Eight women with slightly oedematous ankles volunteered to take part in this study. The mean age, weight, blood pressure taken when the subject sat up as well as the length of pregnancy are recorded in Table I. Four of the mothers had delivered one child and four were nulliparae. None of the mothers suffered from any disease. The course of the pregnancy was uneventful and apart from iron tablets none of them received any treatment. Each mother carried out her ordinary daily routine during the study period.

On the first day of the study the women collected a 4-hour urine specimen starting at 7 a.m. At 1 p.m. a venous blood sample was withdrawn. The following morning when the women brought their first urine samples at 7 a.m. each was given a 500 mg tablet of chlorthiazide (Salutind® Leiras Finland) after which they started the new 24-hour urine collection. At 1 p.m. they came for blood sampling; they also received six more chlorthiazide tablets and were told to take one tablet each subsequent morning. On the 7th day they took the last tablet at 7 a.m. and began to collect the last 24-hour urine sample. At 1 p.m. they had their last blood sample taken. A special diet was not given but the women were instructed not to alter their dietary habits during the study. The women fasted for two hours before blood sampling.

The 5 ml blood sample for PRA was always withdrawn into an EDTA tube chilled in ice water. Plasma was separated at 10 min by refrigerated centrifuge at +4°C and stored at -20°C until analysis. For the PRA the angiotensin I generated by plasma renin in one hour at 37°C at pH 6.0 (maleate buffered) was determined by radioimmunoassay (12) and expressed as ng angiotensin I/ml/hour.

The volume of the 24-hour urine samples were measured and the daily excretions of aldosterone sodium and potassium were determined. Free and acid labile glucuronized aldosterone in the urine were determined by radioimmunoassay by using first the method of Bayard et al. (1) for hydrolysis and extraction and then the method of Ekins et al. (5) for chromatographic purification. Urinary sodium and potassium were determined using an atomic absorption spectrophotometer (Varian Tectron Model 1100). The intra assay coefficient of variation (SD) of 10 samples

Table 1 The subjects participating in the study ($n=8$)

	Mean	S E M	Range
Age (years)	25.3	1.6	20–31
Weight (kg)	77.4	4.0	69–90
Duration of pregnancy (weeks)	31.3	0.7	29–35
Systolic blood pressure (mmHg)	127	3	120–130
Diastolic blood pressure (mmHg)	77	3	70–90

in the same series was 0.38 for PRA at the level of 4.5 ng/ml/h and 5.5 for aldosterone at the level of 65.7 μ g/day. The inter assay precision (SD) for the PRA in 17 different series was 0.15 at the level of 1.14 ng/ml/h.

RESULTS

The PRA (Fig. 1) on the day before treatment was 15.2 ± 1.2 (S E M) ng/ml/h. The level reached 6 hours after the first tablet was already significantly increased ($p < 0.05$). On the last day of the study the level was 31.3 ± 4.8 ng/ml/h. The difference compared to the pre treatment value is significant ($p < 0.01$).

The urinary excretion of aldosterone (Fig. 1) before treatment was 55.9 ± 10.4 μ g/24 hours. In the 24 hours after the first chlorthiazide tablet the excretion was higher ($p < 0.05$) and on the 7th day it was 117.0 ± 9.8 μ g/24 hours, significantly higher than pre treatment excretion ($p < 0.001$). The increase in aldosterone excretion during the first day correlated positively with the corresponding PRA increase (Table II).

The urinary excretion of sodium (Fig. 2) increased from 102 ± 19 mmol/24 hours to 131 ± 21 mmol in the 24 hours after the first tablet ($p < 0.05$) but the excretion of sodium in the 7th day had decreased to 115 ± 11 mmol/24 hours and thus no longer differed from the pre treatment excretion. The higher the pre treatment excretion of sodium the less the excretion increased on the first and the 7th day (Table II). The increase in PRA from the pre treatment to the 7th day was in positive correlation with the corresponding increase of sodium excretion (Table II).

The urinary excretion of potassium did not change significantly during the treatment but a slight tendency to rise is evident (Fig. 2). The increase of potassium excretion before treatment to the first day showed a positive correlation with the

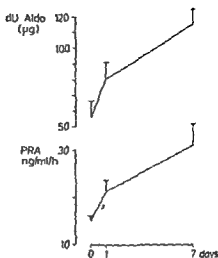


Fig. 1 Mean levels (\pm S E M) of plasma renin activity (PRA) and daily urinary excretion of aldosterone (dU Aldo) before and in the first and seventh day chlorthiazide treatment during pregnancy. $^{**}p < 0.01$, $^{*}p < 0.05$.

increase of aldosterone excretion (Table II). The ratio of excretions of sodium and potassium increased from 2.2 ± 0.2 to 2.8 ± 0.3 in the first day ($p < 0.01$) but decreased to the level of the pre treatment day in the 7th day.

The volume of the 24 hour urine was significantly greater in the 24 hours after the first tablet of chlorthiazide ($p < 0.01$). On the 7th day however the urine excretion was lower ($p < 0.05$) than on the first day of the treatment and did not differ any longer from the pre treatment day excretion (Fig. 2).

DISCUSSION

In our earlier study we found a non pregnant PRA level of 2.2 ± 0.3 ng/ml/h ($n=27$) (17). In the 30th week of pregnancy we found the level of 15.2 ± 1.2 ng/ml/h ($n=10$) which is the same level as we found in this study on the pre treatment day. During the chlorthiazide treatment the PRA level doubled within one week. In non pregnant subjects a similar increase was noticed during the first week of diuretic treatment (16) but the initial levels were considerably lower. In these cases the renin is of renin origin. In pregnancy the PRA level is much higher. It seems unlikely that all the renin is released from the kidneys and production in the placenta and membranes has been suggested (10, 11).

The increased excretion of sodium and cor

Table II Coefficients of linear correlation (*r*) between the diuretic induced changes of PRA, dU-Aldo, dU-Na and dU-K

Abbreviations as in Figures

	dU-Aldo	dU-Na	dU-K
PRA	0.693 (0-1 day)	0.823 (0-7 days)	
dU-Aldo		0.595 (0-1 day)	0.866 ⁺ (0-1 day)
dU-Na		0.870 (0-1 day)	0.923 ⁺ (0-7 days)
dU-K		0.876 (0-7 days)	

decrease in the body exchangeable sodium which leads to a decrease of plasma volume during diuretic treatment might be one possible explanation for the increase of PRA (15). It is likely that this decrease in plasma volume leads to a decrease in placental perfusion and—as a reaction—renin may be liberated from the fetoplacental unit. This renin release could be significant in the maintenance of maternal blood pressure and consequently in the maintenance of the placental perfusion (7).

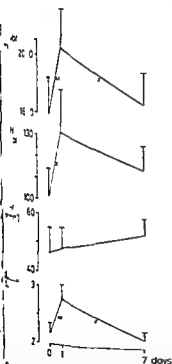


Fig. 2. Mean levels (\pm S.E.M.) of daily urinary excretion of sodium (dU-Na) and potassium (dU-K) and sodium-potassium ratio before and in the first and seventh day of chlorthalidide treatment during pregnancy.

According to the earlier findings presented in one of our studies (12) the aldosterone excretion of non-pregnant women as $3.7 \pm 0.3 \mu\text{g}/24$ hours ($n=27$) and in the 30th week of pregnancy 47.9 ± 13.0 ($n=10$). In this study the aldosterone excretion in the 31st week was 55.9 ± 10.4 on the pre-treatment day but it was also more than double on the 7th day of the chlorthalidide treatment. It is likely that the increase of progesterone level during pregnancy is the stimulator of aldosterone secretion (14). With our high levels of aldosterone the pregnant woman would lose—due to her progesterone alone—all her exchangeable sodium into the urine within about four hours (2).

In this study the addition of exogenic diuretic effect to the progestational effect in the kidneys required a doubled aldosterone secretion; an equally high level is found only in Conn's syndrome. Urine output increased rapidly at the onset of diuretic treatment and then gradually decreased. This has also been observed in non-pregnant subjects (16). The increase in aldosterone secretion could be the result of the increased sodium excretion and mediated by the rise in renin secretion. During pregnancy the hypokalemic effect of saluretics is well established and this can be assumed to be due largely to the increased aldosterone secretion. In this study no significant rise of potassium excretion was detected but the correlation between the increases of aldosterone and potassium excretions was positive.

Clinically it is concluded that after 7 weeks treatment with chlorthalidide the urine output is at the same level as before the treatment. If further increase is needed it probably can be brought about by using aldosterone antagonists. With diuretic treatment the plasma volume decreases at least temporarily while as a reaction PRA rises; this may minimize a possible decrease in the placental blood flow.

Table I The subjects participating in the study ($n=8$)

	Mean	S E M	Range
Age (years)	25.3	1.6	20–31
Weight (kg)	77.4	4.0	69–90
Duration of pregnancy (weeks)	31.3	0.7	29–35
Systolic blood pressure (mmHg)	127	3	120–130
Diastolic blood pressure (mmHg)	77	3	70–90

in the same series was 0.38 for PRA at the level of 4.5 ng/ml/h and 5.5 for aldosterone at the level of 65.7 μ g/day. The inter assay precision (SD) for the PRA in 17 different series was 0.15 at the level of 1.14 ng/ml/h.

RESULTS

The PRA (Fig. 1) on the day before treatment was 1.2 ± 1.2 (S E M) ng/ml/h. The level reached 6 hours after the first tablet was already significantly increased ($p < 0.05$). On the last day of the study the level was 31.3 ± 4.8 ng/ml/h. The difference compared to the pre treatment value is significant ($p < 0.01$).

The urinary excretion of aldosterone (Fig. 1) before treatment was 55.9 ± 10.4 μ g/24 hours. In the 24 hours after the first chlorthiazide tablet the excretion was higher ($p < 0.05$) and on the 7th day it was 117.0 ± 9.8 μ g/24 hours, significantly higher than pre treatment excretion ($p < 0.001$). The increase in aldosterone excretion during the first day correlated positively with the corresponding PRA increase (Table II).

The urinary excretion of sodium (Fig. 2) increased from 102 ± 19 mmol/24 hours to 131 ± 21 mmol in the 24 hours after the first tablet ($p < 0.05$) but the excretion of sodium in the 7th day had decreased to 115 ± 11 mmol/24 hours and thus no longer differed from the pre treatment excretion. The higher the pre treatment excretion of sodium the less the excretion increased on the first and the 7th day (Table II). The increase in PRA from the pre treatment to the 7th day was in positive correlation with the corresponding increase of sodium excretion (Table II).

The urinary excretion of potassium did not change significantly during the treatment but a slight tendency to rise is evident (Fig. 2). The increase of potassium excretion before treatment to the first day showed a positive correlation with the

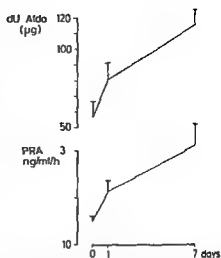


Fig. 1 Mean levels (\pm S E M) of plasma renin activity (PRA) and daily urinary excretion of aldosterone (Aldo) before and in the first and seventh day chlorthiazide treatment during pregnancy. $^{*}p < 0.01$, $^{**}p < 0.001$.

increase of aldosterone excretion (Table II). The ratio of excretions of sodium and potassium increased from 2.2 ± 0.2 to 2.8 ± 0.3 in the first day ($p < 0.01$) but decreased to the level of the pre treatment day in the 7th day.

The volume of the 24 hour urine was significantly greater in the 24 hours after the first tablet chlorthiazide ($p < 0.01$). On the 7th day however the urine excretion was lower ($p < 0.05$) than on the first day of the treatment and did not differ any longer from the pre treatment day excretion (Fig. 2).

DISCUSSION

In our earlier study we found a non pregnant PRA level of 2.2 ± 0.3 ng/ml/h ($n=27$) (13). In the 10 week of pregnancy we found the level of 15.1 ± 1.1 ng/ml/h ($n=10$) which is the same level as we found in this study on the pre treatment day. During the chlorthiazide treatment the PRA level doubled within one week. In non pregnant subjects a similar increase was noticed during the first week of diuretic treatment (16) but the initial levels were considerably lower. In these cases the renin is of renin origin. In pregnancy the PRA level is much higher. It seems unlikely that all the renin is released from the kidneys and production in the placenta membranes has been suggested (10, 11).

The increased excretion of sodium and co-

Table II Coefficients of linear correlation (r) between the diuretic induced changes of PRA, dU -Aldo, dU -Na and dU -K⁺

Abbreviations as in Figures

	dU -Aldo	dU -Na	dU -K
IA	0.693 (0-1 day)	0.823 (0-7 days)	
Aldo		0.595 (0-1 day)	0.876 (0-1 day)
Na		0.870 (0-1 day)	0.923 (0-7 days)
K		0.876 (0-7 days)	

or a decrease in the body exchangeable sodium which leads to a decrease of plasma volume during diuretic treatment might be one possible explanation for the increase of PRA (15). It is likely that this decrease in plasma volume leads to a decrease in placental perfusion and—as a reaction—renin may be liberated from the fetoplacental unit. Thus renin release could be significant in the maintenance of maternal blood pressure and consequently in the maintenance of the placental perfusion (7).

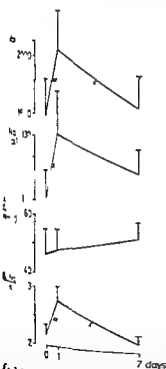


Figure 2 Mean levels (\pm S.E.M.) of daily urinary excretion of sodium (dU -Na) and potassium (dU -K) and sodium-potassium ratio before and in the first and seventh day of chlorthiazide treatment during pregnancy

According to the earlier findings presented in one of our studies (12) the aldosterone excretion of non pregnant women as $3.7 \pm 0.3 \mu\text{g}/24$ hours ($n=27$) and in the 30th week of pregnancy 47.9 ± 13.0 ($n=10$). In this study the aldosterone excretion in the 31st week was 55.9 ± 10.4 on the pre treatment day but it was also more than double on the 7th day of the chlorthiazide treatment. It is likely that the increase of progesterone level during pregnancy is the stimulator of aldosterone secretion (14). With out high levels of aldosterone the pregnant woman would lose—due to her progesterone alone—all her exchangeable sodium into the urine within about four hours (2).

In this study the addition of exogenic diuretic effect to the progestational effect in the kidneys required a doubled aldosterone secretion; an equally high level is found only in Conn's syndrome. Urine output increased rapidly at the onset of diuretic treatment and then gradually decreased. This has also been observed in non pregnant subjects (16). The increase in aldosterone secretion could be the results of the increased sodium excretion and mediated by the rise in renin secretion. During pregnancy the hypokalemic effect of saluretics is well established and this can be assumed to be due largely to the increased aldosterone secretion. In this study no significant rise of potassium excretion was detected but the correlation between the increases of aldosterone and potassium excretions was positive.

Clinically it is concluded that after a week's treatment with chlorthiazide the urine output is at the same level as before the treatment. If further increase is needed it probably can be brought about by using aldosterone antagonists. With diuretic treatment the plasma volume decreases at least temporarily while as a reaction PRA rises this may minimize a possible decrease in the placental blood flow.

Table I The modified Bishop score used in the present investigation

	Score rating		
	0	1	2
Cervical dilatation	<0.5	0.5-1.5 cm	>1.5 cm
Cervical effacement	None	<50%	>50%
Station of fetal head	Above or in pelvic inlet	Above spines	At or below spines
Cervical consistency	Firm	Medium	Soft
Cervical position	Posterior	Medium	Anterior

gestational age (Table III). None of the patients had suspected cephalopelvic disproportion or breech presentation. All had intact membranes.

Preparation of PGE₂-gel

A solution of PGE₂ was prepared by mixing PGE₂ powder (Upjohn) in ethanol. The solution was sterile filtered and kept at -20°C. The viscous medium was prepared by mixing 3.0 g hydroxypropylmethyl cellulose with 100 ml hot water. The mixture was autoclaved at 120°C for 20 minutes. After cooling and shaking the viscous medium was divided into 5 ml portions and stored in small glass containers. The clinically used PGE₂ gel was prepared immediately before use by mixing 0.2 ml PGE₂ solution (1.0 mg) with 4 ml of the viscous medium.

Experimental procedure

All patients had ultrasound scanning of the uterus performed before the investigation was started. By this procedure the localization of the placenta, the configuration of the uterus and the size and position of the fetus were determined. Patients with low implanted placentas were excluded from the study. On the morning of the day before planned induction the cervical state was determined always by the same investigator according to a modified Bishop score (Table I). With the patient in the lithotomy position the PGE₂ gel was administered intracervically via a 17 cm long catheter with an outer diameter of 3.3 mm. The gel was placed as far as possible into the cervical canal—as a rule close to the internal os. After application of the gel the catheter was withdrawn and the patient was requested to stay in bed for 2 hours.

All patients remained under supervision in the antenatal

ward. Pulse and blood pressure were recorded intermittently. The patients were asked to comment on the effect of the treatment including possible side effects. In most patients uterine contractions were monitored by external tocometry (Hewlett Packard or Roche). If the treatment induced regular myometrial contractions affecting the cervical state the patients were moved to the delivery ward and supervised using routine techniques such as external or internal cardio-tocography and repeated measurements from the fetal scalp. In the patients whom labour was not induced or if the contractions waned the cervical state was reassessed the following morning before the induction of labour with intravenous oxytocin.

RESULTS

Group A

All 41 patients experienced uterine contractions within half an hour after PGE₂ gel administration. Twenty three (56%) progressed without further stimulation into established labour within 6 hours. In the mean delivery occurred after 10 hours (range 4 to 15 hours).

Table II gives the effect on the cervical state of the 18 patients of group A in whom uterine contractions did not progress into established labour. The Bishop score changed during 24 hours from

Table II Results of intracervical instillation of 1 ml PGE₂ in the patients of Group A

	Delivered after primary induction (n=73)	Undelivered after primary induction (n=18)
Nulliparae	10	10
Multiparae	13	8
Bishop score before instillation	3.3 (1-5)	2.5 (0-4)
Bishop score after instillation	-	6.1 (4-8)

Table III Clinical data of the patients in Group A and results of the double blind study

	Gel with placebo (n=10)	Gel with PGE ₂ (n=10)
Nulliparae	10	10
Mean age	25 (18-33)	24 (18-35)
Mean gestational age (weeks)	41 (39-43)	40 (39-41)
Mean Bishop score before instillation	3.6 (2-5)	3.5 (2-5)
Mean Bishop score 24 hours after instillation	4.0 (2-6)	6 and 7 (7 patients)
Delivered after primary induction	8	8

0 to 4) to 6 l (range 4 to 8). Fourteen of the 18 women had labour induced successfully with an intravenous oxytocin infusion the day after PGE₂ administration. The time to delivery was 8 hours (4 to 17 hours). Concerning the other 4 patients 1 was delivered after a second infusion with intravenous oxytocin and 1 went into spontaneous labour 2 days after the first trial. In 2 patients the membranes ruptured spontaneously 2 days after the first induction they were successfully stimulated with intravenous oxytocin and delivered uneventfully.

Group B

All patients who were given active gel (the gel containing 10 mg PGE₂) experienced uterine contractions within half an hour. In 8 patients the contractions progressed to established labour and they were delivered without further stimulation within 10 hours (range 5 to 13 hours) after the application of the gel. In 2 patients contractions waned after about 3 to 4 hours.

The cervical state changed from a Bishop score of 1 to 6 and 7 respectively during 24 hours. In these 10 patients labour was induced the morning after PGE₂ administration by means of oxytocin infusion and delivery occurred successfully after 8 and 10 hours.

In the 10 patients receiving placebo gel (gel without PGE₂) only 2 experienced uterine contractions within half an hour. The contractions disappeared completely after about 2 hours. At examination the next morning before induction the mean Bishop score was 4.0 compared with 3.6 before the application of the gel. In the remaining 8 patients no contractions or consistent change in Bishop score were noticed during 24 hours (Table III).

Three patients receiving placebo gel were induced successfully with intravenous oxytocin the day after the gel administration. The time to delivery was 10, 13 and 17 hours. In the remaining 7 patients the first oxytocin induction failed. However all were successfully induced and delivered within 1 week of the trial.

In group A 5 patients (12%) were delivered by vacuum extraction because of fetal distress in the second stage of labour. Four of the children had 5 minute Apgar scores of 8 to 10 but the remaining child had an Apgar score of 3. There were no problems at the induction of this patient who went into established labour 3 hours after application of the gel. She was supervised routinely in the delivery

ward and labour was normal until late second stage. Then a marked fetal bradycardia occurred and a vacuum extraction was performed. At delivery the child had a severe bradycardia and was cyanotic and hypotonic. The child died after 2 hours despite intubation and ventilation with oxygen. The subsequent autopsy revealed intracranial hemorrhage.

All patients receiving active PGE₂ gel in group II were delivered vaginally. The children had 5 minute Apgar scores of 9 or 10. Four patients in group II receiving placebo gel had to be instrumentally delivered 3 by Caesarean section because of prolonged labour and 1 by vacuum extraction because of fetal distress in the second stage of labour. The infants of the patients receiving placebo gel had 5 minute Apgar scores of 8 to 10.

In all patients in whom uterine contractions progressed into established labour and in whom delivery occurred without further stimulation there was no need for analgesia. No signs of uterine hypertonus or abnormal myometrial activity were observed. There were no complaints of gastrointestinal discomfort and no marked changes in pulse rate or blood pressure were recorded. In one patient there was a transient increase in temperature from 37.5°C to 38.2°C. This patient also had a short period of shivering.

DISCUSSION

Priming or ripening of the uterine cervix before induction of labour by means of PGE₂ in a viscous medium applied locally has recently been reported by several investigators e.g. Shepherd et al (1976), Calder et al (1977), Thiery et al (1977) and MacKenzie & Embrey (1977). The doses used when administering the prostaglandin extraamniotically have generally ranged between 250 and 500 µg. Calder et al (1977) suggested that the optimum priming dose of PGE₂ is about 400 µg. However because of failure of induction or unimproved cervical state they considered that a larger dose might have been more effective in some patients. Twenty four of their 121 patients (20%) were either delivered or established in labour on the morning after PGE₂ administration. In the remaining 97 patients the cervical score had improved from a mean of 2.3 to 6.3.

In the present investigation a dose of 1 mg was deposited intracervically. This resulted in a higher frequency of successful inductions with PGE₂ than

in the study of Calder et al (1977) 31 of 51 patients (61%) were delivered within 15 hours. The cervical improvement in undelivered patients and the frequency of Caesarean section were similar. It is probable that the difference in the incidence of successful induction is related to the difference in dose. The frequency of fetal distress in the second stage of labour (5 in 51 patients) in the present study might also have some relation to the dose. Even though no signs of uterine hypertonus occurred in our patients a smaller dose might have been sufficient. Like Calder et al (1977) we feel that the dosage could be adjusted to the cervical score.

Passing a catheter through the cervical canal to deposit viscous medium was demonstrated to have a negligible effect on the state of the cervix by Shepherd et al (1976). The present results fully confirmed this finding, as the patients receiving gel without PGE₂ showed no consistent changes in their cervical state.

The mode of action of the locally administered PGE₂ on cervical tissue is not known. According to Danforth et al (1974) cervical dilatation during labour is an active process involving changes in cervical composition. They found that in the cervix immediately after delivery there was a slight increase in water, a marked decline in collagen and glycoprotein and a marked increase in glycosaminoglycans. In addition a new component of unidentified nature was isolated. Using an in vitro model Conrad & Ueland (1976) found that PGE₂ reduced the cervical connective tissue stiffness. They investigated only the dense collagenous core of the cervix which excluded effects on the muscle layer. Studies by Coutinho & Darzé (1976) on cervical mechanical activity in vivo suggest that PGE₂ has a direct relaxing effect of the smooth muscle of the non pregnant uterine cervix. This agrees with in vitro studies by Najak et al (1970) which showed that spontaneous contractile activity of the cervical muscle layer was increased by PGF₂ and decreased by PGE₂.

The present study confirms previous reports that local administration of PGE₂ in a viscous medium can induce ripening of the cervix and may some times induce labour in patients reaching term with an unfavourable cervical state.

REFERENCES

1 Calder A A & Embrey M P Prostaglandins and the unfavourable cervix. *Lancet* 2 1372 1973

2 Calder A A Embrey M P & Tait T Ripening the cervix with extra amniotic prostaglandin E viscous gel before induction of labour. *Br J Ob Gynaecol* 84 764 1977

3 Conrad J T & Ueland K Reduction of the stre modulus of human cervical tissue by prostaglan E₂. *Am J Obstet Gynecol* 126 218 1976

4 Coutinho M & Darzé E Spontaneous contractility and the response of the human uterine cervix prostaglandins F₂ and E₂ during the menstrual cycle. *Am J Obstet Gynecol* 126 774 1976

5 Danforth D N Veis A Breen M Weinstein G Buckingham J C & Manalo F The effect pregnancy and labor on the human cervix. Change collagen glycoproteins and glycosaminoglycans. *J Obstet Gynecol* 120 641 1974

6 Embrey M P In The Management of Labour Proceedings of the Third Study Group of the R C O (ed R W Beard M Brudenall P Dunn & D F weather) p 67 Royal College of Obstetricians Gynecologists London 1975

7 Embrey M P Hillier K & Mahendran P Induction of abortion by extra amniotic administration prostaglandins E₂ and F₂. *Br Med J* 3 146 1971

8 Hendricks C H Brenner W E & Kraus G H Maternal cervical dilatation in late pregnancy and lab. *Am J Obstet Gynecol* 106 1065 1970

9 Karim S M M & Sharma S D Second trimester abortion with single intra amniotic injection prostaglandins E₂ or F₂. *Lancet* 2 47 1971

10 Mackenzie I Z & Embrey M P Cervical ripening with intravaginal prostaglandin E₁ gel. *Br Med J* 2 1381 1977

11 Miller A W F Calder A A & Macnaughton C Termination of pregnancy by continuous intrauterine infusion of prostaglandins. *Lancet* 2 1972

12 Miller A W F & Mack I S Induction of Labour by extra amniotic prostaglandins. *J Obstet Gynaecol Br Commonw* 81 706 1974

13 Najak Z Hillier K & Karim S M M The effect of prostaglandins on the human isolated non pregnant cervix. *J Obstet Gynaecol Br Commonw* 77 1970

14 Shepherd J Sims C & Craft K Extra-amniotic prostaglandins E₂ and the unfavourable cervix. *Lancet* 2 709 1976

15 Thiery M Defoort G Benjts J Van Eyck Hennay T Van Kets H & Martens C Effectiveness of extra-ovular injection of prostaglandin E₂ in viscous gel in ripening the cervix prior to elective induction of labor at term. *Prostaglandins* 14 381 1977

16 Turnbull A C & Anderson A B M Induction of labour Part II Intravenous oxytocin infusion. *J Obstet Gynaecol Br Comm*

Submitted for publication Oct 75 1977

Ulf Ulmsten
Dept of Obstetrics and Gynaecology
University Hospital
S 21401 Malmö Sweden

INTRAUTERINE SUPRAVENTRICULAR TACHYCARDIA

N. H. Valerius and J. Ramsøe Jacobsen

From the Department of Paediatrics TG Queen Louise's Hospital for Children and Department of Neonatology GN Rigshospitalet University of Copenhagen Denmark

Four cases of intra uterine ectopic supraventricular tachycardia are described. In three there were none or only minor symptoms immediately before delivery and subsequently. The fourth baby having a neonatal W-P-W syndrome was born with severe hydrops fetalis and was asphyxiated. From these cases and from data reported in the literature it is concluded that intra uterine heart failure is a significant risk when the fetal heart rate persistently exceeds 230 per minute. It is suggested that digoxin administered to the mother may be beneficial in the fetus in cases of intra uterine ectopic tachycardia.

Less than 50 cases of intrauterine supraventricular tachycardia (IT) have been reported. In a review Linkoff collected 17 cases and he concluded that IT was most often well tolerated by the fetus (18). However, several recent reports have stressed that IT may result in severe intrauterine cardiac failure in babies being born with hydrops fetalis or developing severe cardiorespiratory distress immediately after birth (11, 22, 27). It is the purpose of the present communication to review the identification of the cases of IT at risk of developing intrauterine complications from a review of the literature and from the observations in new cases.

CASE REPORTS

Case I. A girl born 1966. Four weeks before term the fetal heart rate was found increased by auscultation. One week before term a fetal ECG demonstrated a regular tachycardia of 80/min. As the tachycardia persisted a caesarean section was performed 5 days later. The baby was severely depressed with an Apgar score of 5 at 1 min but 9 at 5 min. Birth weight 3450 g. An ECG confirmed the presence of supraventricular tachycardia of 200/min (16). During the first 3 days of life the tachycardia became repetitive and alternating with short episodes of sinus rhythm. The tachycardia continued until the age of 13 months and caused no symptoms. This patient was previously reported elsewhere (16).

Case II. A boy born 1977. 3½ months prior to term a

fetal tachycardia of 240/min was noted by the home physician. During the remaining part of the pregnancy the fetal heart rate was consistently observed to alternate between 74 and 140 per min. The delivery was spontaneous and uncomplicated at term. The baby was well with an Apgar score at 10 at 1 min. Birth weight 3100 g. An ECG showed short sequences of sinus rhythm of 160/min alternating with paroxysms of supraventricular tachycardia of 240/min lasting from 10 to 60 sec. Due to mild tachypnea digoxin was administered from the second day of age followed by gradual disappearance of the tachycardia. Digoxin was discontinued at 7 days of age but resumed as two isolated paroxysms were observed three days later. No further tachycardia was noted not even after discontinuation of the treatment at the age of 6 months.

Case III. A boy born 1973. Two weeks prior to term the mother had a routine examination at hospital and nothing abnormal was disclosed. During the following two days the mother noticed absence of fetal movements and contacted the hospital. A fetal tachycardia of 248/min was observed. It persisted for the following 4 hours and caesarean section was performed on the suspicion of fetal distress. The delivery was difficult and the baby was severely asphyxiated with an Apgar score of 3 at 1 and 3 min and 7 at 15 min. There was no tachycardia. During vigorous resuscitation several episodes of bradycardia and asystole were observed until the heart rhythm settled at 140/min at 15 min of age. Birth weight 3850 g. There was extensive oedema including ascites, hydrothorax and pulmonary rales. The infant was treated in an incubator with oxygen, digoxin and frusemide. The blood sugar was found to be zero and was corrected with intravenous glucose. The ECG (Fig. 1) revealed sinus rhythm with Wolf-Parkinson-White syndrome (WPW) or pre-excitation type A. An anomaly frequently associated with paroxysmal supraventricular tachycardia. No blood group incompatibility was revealed. At 8 hours the supraventricular tachycardia recurred at a rate of 250/min. Practolol 1 mg i.v. had no effect. As the blood sugar was low again 0.7 mmol/l sampled prior to the administration of practolol glucose was given i.v. and during the injection the tachycardia reverted into sinus rhythm. The infant lost 780 g during the first three days of life. He presented no further cardiac problems but the course was complicated by fits and cerebral hemorrhage. At the age of one year there were signs of spastic diplegia, blindness and mental retardation. The ECG demonstrated WPW syndrome until the age of 10 weeks and later only normal sinus rhythm.

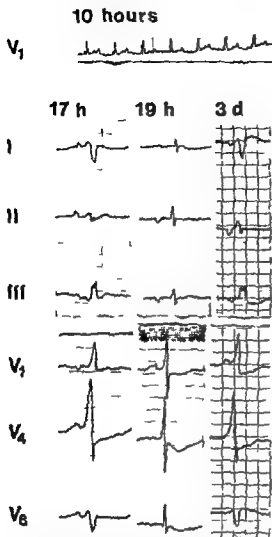


Fig. 1 ECG in case III showing atrial tachycardia of 250 per min at 10 hours, sinus rhythm with preexcitation at 17 hours, ectopic (left) atrial rhythm with normal ventricular activation at 19 hours, and preexcitation at 3 days of age.

ever since the age of 6 months he has had recurrent paroxysmal supraventricular tachycardia despite treatment.

Case IV. A girl born 1975. The mother was admitted to hospital at term because of decreasing estriol excretion. The fetal heart rate was 136/min. On the following day the mother noticed absence of fetal movements and a fetal tachycardia of 260/min was revealed. A few hours later fetal movements returned, but the fetal heart rate was not recorded. Two days later a caesarean section was performed because of a breech presentation and a maternal age of 40. The baby was well at birth but the heart rate was found to be 300/min. An ECG confirmed the presence of supraventricular tachycardia. Digitalization was instituted and sinus rhythm returned within the next 24 hours. The ECG now demonstrated WPW syndrome. The baby remained in excellent condition until the age of 7 weeks when she was admitted after a syncope. Continuous ECG

monitoring in hospital revealed intermittent atrial arrhythmia and atrioventricular conduction delay suggesting digitalis toxicity. Digoxin was withdrawn; she had no further symptoms. From the age of 3 years she has had normal sinus rhythm without WPW syndrome.

DISCUSSION

The four cases demonstrate the variable outcome of IT. While cases I, II and IV had none or very few symptoms only immediately after birth, case I presented with hydrops fetalis and severe asphyxia probably as the result of intrauterine failure. This patient and patient IV also had W-P-W syndrome which has been reported on once previously in a case of IT (11). There are previous reports of IT associated with hydrops fetalis (22, 27) and in another three cases of hydrops (5, 14, 17) an ectopic tachycardia was observed immediately after birth. One patient with hydrops fetalis from an unknown cause developed paroxysmal atrial tachycardia at 17 days of age (6) and it was suggested that the hydrops had been caused by IT. IT should thus be listed among possible causes in cases of unexplained fetal hydrops.

The development of heart failure in paroxysmal atrial tachycardia in infants is commonly considered related to the extremely high heart rates usually encountered (i.e. 250–300 per minute) and the duration of the paroxysms. The heart rates may have most often been reported to be variable, due to an intermittent tachycardia or to a "2:1" ventricular block, typically seen in atrial flutter, but also in some cases of atrial tachycardia. As an intermittently normal or even reduced heart rate may lead to some recovery from heart failure, the heart rates reported in 46 cases of IT (1–4, 7, 13, 18–22, 24–31) were analyzed for a possible relation to the immediate symptoms at birth. All 6 patients with heart rates persistently above 300 per minute had severe symptoms at birth with an Apgar score less than 7 at 1 min and presenting with either hydrops (27), moderate edema and cyanosis (11) or severe respiratory distress (11, 18, 31). Of the remaining 30 patients 6 had severe symptoms on presenting with hydrops (77) and the remaining 24 with edema, cyanosis and tachypnoea (11, 13, 19). In three of these (12, 13, 19) the lowest reported heart rate was above 200 per minute.

The duration of the tachycardia cannot be of

prostatic importance as it will usually be unknown at the time of diagnosis. Thus the observed duration of IT in three patients presenting with hydrops ranged between minutes (22) and 33 days (27) and was probably 2 days in our case III. Eight cases in addition to our case (no. II) had IT for more than 1 month (7, 8, 9, 17, 26-29) and only one of these cases (27) had symptoms at birth.

Before the introduction of electroconversion digitalis was the main treatment of paroxysmal tachycardia in infants usually alleviating the constant heart failure and possibly promoting conversion to sinus rhythm. Digitalis is also commonly used for the prevention of recurrences. Digoxin readily crosses the placenta, the serum level in cord blood being identical to the maternal serum level when in maintenance treatment (23). Toxic serum levels are higher in infants than in adults. Thus it is very likely that digitalization of the mother aimed at the therapeutic serum level of 0.5-1.5 ng/ml will be of no harm and of possible benefit to the fetus having IT. No such therapeutic approach has been reported in the literature but it deserves consideration in those cases in which it is decided to terminate the pregnancy.

From the four cases reported here and from the data obtained from the literature it can be concluded that IT is most often well tolerated. There is, however, a risk of intrauterine heart failure when the fetal heart rate persistently exceeds 200/min and the risk becomes high when the heart rate rises above 230/min. It does not seem warranted to terminate the pregnancy at least prior to term when the heart rate is lower than 200/min in the absence of other signs of fetal distress. Irrespective of a good condition immediately after birth the infants with continuing tachycardia may develop overt heart failure within hours or days requiring immediate paediatric attention. Delivery of babies with IT should therefore be performed where intensive neonatal care is available.

REFERENCES

1. A. J. Laitinen I & Simola S. Congenital atrial flutter. *Acta Paediatr Scand* 59: 587 1970.
2. Bernstein R L, Winkler J E & Callagan III A. Fetal bigeminy and tachycardia. *Am J Obstet Gynecol* 101: 856 1961.
3. Blumenthal S, Jacobs J, Steer C M & Wil-
lamson S W. Congenital atrial flutter. Report of a case documented by intra-uterine electrocardiogram. *Pediatrics* 41: 659 1968.
4. Carr J G & McClure W B. Auricular flutter in a newly born infant. *Am Heart J* 6: 874 1931.
5. Cowan R H, Waldo A L, Harns H B, Cassidy III & Brans Y W. Neonatal paroxysmal tachycardia with hydrops. *Pediatrics* 55: 4: 8 1975.
6. Dyggve H. Hydrops foetalis without blood group incompatibility but associated with hydramnios. *Acta Paediatr Scand* 49: 437 1960.
7. Frisell E. Zur Frage der paroxysmalen Tachycardie und des Herzflatters in den ersten Lebenswochen. *Acta Paediatr Scand* 34: 30 1947.
8. Garvin J A & Kline E M. Congenital paroxysmal tachycardia. *Am Heart J* 33: 367 1947.
9. Hassenruck A, Chojnacki B & Barker H J. Cardioversion of auricular flutter in a newborn infant. *Am J Cardiol* 15: 776 1965.
10. Hedberg H T. Foetal arrhythmia—congenital auricular flutter. *Acta Obstet Gynecol Scand* 25: 392 1945.
11. Hedvall G. Congenital paroxysmal tachycardia. A report of three cases. *Acta Paediatr Scand* 62: 550 1973.
12. Herin P & Thoren C. Congenital arrhythmias with supraventricular tachycardia in the perinatal period. *Acta Obstet Gynecol Scand* 52: 381 1973.
13. Hiltnich N M & Evrard J R. Supraventricular tachycardia in the newborn with onset in utero. *Am J Obstet Gynecol* 70: 1139 1955.
14. Horst R L v d. Congenital atrial flutter and cardiac failure presenting as hydrops foetalis at birth. *S Afr Med J* 44: 1037 1970.
15. Hung W & Walsh II J. Congenital auricular fibrillation in a newborn infant with endocardial fibroelastosis. *J Pediatr* 61: 65 1962.
16. Jacobsen J, Ramsøe Andersen E D, Sandøe E, Videbæk J & Wennevoeld A. Chronic supraventricular tachycardia in infancy and childhood. *Acta Paediatr Scand* 64: 597 1975.
17. Kesson C W. Foetal paroxysmal auricular fibrillation. *Br Heart J* 20: 557 1958.
18. Levkoff A H. Perinatal outcome of paroxysmal tachycardia of the newborn with onset in utero. *Am J Obstet Gynecol* 104: 73 1969.
19. Levy D L. Persistent foetal tachycardia in utero prior to labour in an infant with congenital cytomegalic inclusion disease. Case report. *Am J Obstet Gynecol* 112: 859 1977.
20. McDonagh II J. Congenital atrial flutter. *Arch Dis Child* 43: 731 1968.
21. Müller Schmid F. Die paroxysmale Tachycardie in utero. *Geburtsh Frauenheilk* 19: 401 1959.
22. Pande H. Intrauterine tachycardia og hydrops foetalis. 4. Nordiske Kongres for Perinatal Medicin. København 1973.
23. Rogers M C, Willerson J T, Goldblatt A & Smith T W. Serum digoxin concentrations in the human fetus, neonate and infant. *N Engl J Med* 287: 1010 1972.
24. Sancetta S M, Redding T H & W.

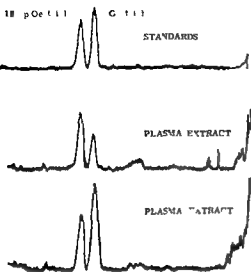


Fig 1

oestrol were plotted and the results gave a straight line passing through the origin (Fig 2). Monitoring of the *m/e* 50 ion of oestrol trimethylsilyl ether gave a greater response than 16-epi oestrol for a given weight.

Reproducibility

In order to investigate the reproducibility two maternal serum samples were analysed five times. The results (ng/100 ml) were as follows: Sample 1 10.6, 1.99, 10.3, 10.3; Sample 2 18.5, 18.0, 19.0, 18.7. The coefficient of variations was 8.3% for Sample 1 and 6.5% for Sample 2.

Oestrol levels in maternal and umbilical cord plasma

The following results for plasma oestrol in maternal arm vein and cord vein plasma in normal and small for dates cases were obtained.

	Oestrol levels ($\mu\text{g}/100\text{ ml}$)	
	Normal	Small for dates
Number of patients	40	15
Mean maternal plasma oestrol	12.7 ± 5	10.8 ± 2.9
Mean cord vein plasma oestrol	123.37 ± 37	97.0 ± 24.0

Determination of oestrol levels in scalp blood

In the series of normal and small for dates patients fetal scalp capillary blood was taken for determination of pH. It was considered of interest to monitor the oestrol concentration in pooled samples of this blood remaining after the pH estimation. It was impractical to determine oestrol in individual samples and the determination was carried out in duplicate on two separate pools obtained from the normal and small for dates groups of cases. The oestrogens

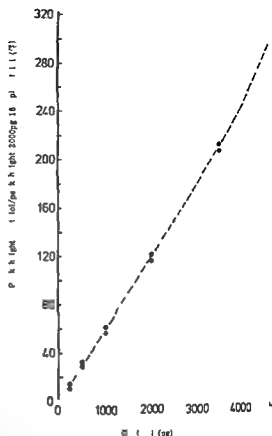


Fig 2 Relative response of oestrol to 16-epioestrol in the range 0–1000 PG

were extracted from whole blood (2 ml) by sonication twice with 5 ml acetone:ethanol 1:1 for 30 min. The ethyl acetate extracts were dried and the steroid compounds hydrolysed in similar fashion in umbilical cord plasma.

The mean amount of oestrol present in the samples of normal scalp capillary blood was $180\text{ }\mu\text{g}/100\text{ ml}$ and in small for dates $165\text{ }\mu\text{g}/100\text{ ml}$. If it is assumed that plasma accounts for half the volume of whole blood then the mean concentration of oestrol in fetal scalp plasma for our series is about $90\text{ }\mu\text{g}/100\text{ ml}$ in normal cases and $82.5\text{ }\mu\text{g}/100\text{ ml}$ for small for dates cases.

DISCUSSION

In the clinical care of women during pregnancy there is extensive literature to show that serial estimates of oestrol are a valuable guide to placental function and fetal wellbeing. In this respect close attention has been given to lowering perinatal mortality and morbidity in common pregnancy complications such as pre-eclampsia (7, 8, 16) and retarded fetal growth and development (3, 21). Active treatment by intermittent abdominal decompression was

produced for pre eclampsia (5) and for small for dates cases (11) with improvement in placental hormone metabolism and fetal mortality rates confirmed by later work (12-13) and by the additional use of sonar increase in fetal growth and viability as shown to occur (20).

Estimation of oestriol has in the past been made from 24-hour urine collections but parallel values have been reported for plasma specimens and the use of radioimmunoassay described (10) and the advantages of this method evaluated both in normal and abnormal pregnancy (9-14).

In the present study with a view to learning more about the distribution of oestriol we investigated its levels in the maternal peripheral vein, the fetal scalp and the baby's cord. We found that the mean oestriol values for maternal plasma during the second stage of labour and cord plasma at birth were both lower in small-for-dates cases compared with normal cases. The mean cord plasma oestriol at $137 \pm 37 \mu\text{g}/100 \text{ ml}$ for normal cases compares well with a level of $129 \pm 44 \mu\text{g}/100 \text{ ml}$ previously reported (1) but Roy (15) recorded lower levels. We also found that the mean oestriol concentration in umbilical blood was ten times that in the relative maternal blood specimens for both normal and small-for-dates cases. In Roy's series there was a similar tenfold increase in concentration in the baby's cord specimens compared with the levels in the mother. Shearman and co-workers (17) reported that the umbilical vein oestriol levels were significantly higher than in the relative maternal plasma. As regards fetal capillary scalp blood just before delivery it was found that there was a higher concentration of oestriol in normal compared with cases of small-for-dates but this was not significant. Most of the recent determinations of plasma oestriol have been as mentioned carried out by saturation techniques but the potential use of gas chromatography-mass spectrometry is now being investigated. Aldercreutz and associates (2) have demonstrated the use of mass fragmentography for the simultaneous measurement of several pregnancy oestrogens in plasma, bile and urine during pregnancy. Taylor & Shackleton (18-19) have employed this technique for the identification of new oestriols. This technique may be available only in specialised laboratories and its routine use is at present more expensive and time consuming than radioimmunoassay methods. Twenty minutes were required for each chromatographic run. Mass

fragmentography does however offer an accurate and reproducible method for plasma oestriol assays and has the advantage in its high specificity and ease of adaptation to determine simultaneously a variety of compounds. Only those compounds with a MW of 504 can interfere with the assay and since other oestriols are present only in small amounts they do not constitute a problem. It was further shown that use of an oestriol epimer as internal standard results in good reproducibility of assay and a correction for procedural losses. No correction was possible for variation in the efficiency of hydrolysis but the technique used was described and validated by Corker & Naftolin (6) from whom stems the recent use of oestriol plasma analysis by radioimmunoassay methods.

Recently other communications on the mass spectrometric analysis of oestrogens have been published. Aldercreutz and colleagues (2) developed a mass fragmentographic method for the simultaneous determination of several oestrogens in plasma, bile and urine of pregnant women. The internal standards used were oestrogen tri-methylsilyl ethers prepared with deuterium labelled reagents which were added to the sample immediately prior to gas chromatographic mass spectrometric analysis. No correction was therefore possible for losses occurring during oestrogen extraction from the body fluids. GC-MS techniques have been shown to be particularly useful for validation of routine methods of oestrogen analysis. Bjorkem and his co-workers (4) measured urinary oestriol by mass fragmentography using an internal standard of ($2\text{-}^3\text{H}_2$) oestriol and compared the results to their routine laboratory method based on a Kober reaction and also to a gas chromatographic method. A good correlation was obtained between all three techniques. Undoubtedly mass fragmentography will be increasingly used for validation of routine steroid determinations. Several new urinary oestriol and oestriol one epimers have recently been identified by GC-MS in this laboratory (18-19) and present research is directed to the measurement of these steroids by selected ion recording in normal and abnormal pregnancies.

ACKNOWLEDGEMENT

We wish to thank Miss S. M. Bekhit FRCS MRCOG for her help in the collection of the various specimens.

REFERENCES

- 1 Atken E II, Preedy J R, K. Eton B & Short R V. Oestrogen and progesterone levels in foetal and maternal plasma at parturition. *Lancet* 2 1096 1958
- 2 Adlercreutz H, Tikkanen M J & Hunneman B H. Mass fragmentographic determinations of eleven estrogens in the body fluids of pregnant and non-pregnant subjects. *Steroid Biochem* 5 211 1974
- 3 Beischer N A, Bhargava V L, Brown J B & Smith M A. The incidence and significance of low oestrol excretion in an obstetric population. *J Obstet Gynaecol Br Commonw* 75 1074 1968
- 4 Bjorkem I, Blomstrand R, Grensson L, Tietz F & Carlstrom K. Validation of methods for determination of urinary oestrol during pregnancy using mass fragmentography. *Clin Chem Acta* 67 385 1975
- 5 Blecher J A & Heyns D S. Abdominal decompression in the treatment of the toxemia of pregnancy. *Lancet* 2 621 1967
- 6 Corker C S & Naftolin F. Rapid method for the measurement of oestrol in pregnancy plasma by competitive protein binding analysis. *J Obstet Gynaecol Br Commonw* 78 330 1971
- 7 Coyle M G & Brown J B. Urinary excretion of oestrol during pregnancy. *J Obstet Gynaecol Br Commonw* 70 225 1963
- 8 Green J W & Touchstone J C. Urinary oestrol as an index of placental function. *Am J Obstet Gynecol* 85 1 1963
- 9 Klopfer A, Janial V & Wilson G. Plasma steroid assay in the assessment of foeto-placental function. *J Steroid Biochem* 6 651 1975
- 10 Macourt D, Corker C S & Naftolin F. Plasma oestrol in pregnancy. *J Obstet Gynaecol Br Commonw* 78 335 1971
- 11 MacRae D J & Mohamedally S M. Effect of abdominal decompression on the metabolism of the foeto-placental unit. *Proc Roy Soc Med* 63 502 1970
- 12 MacRae D J, Mohamedally S M & Willmott M P. Clinical and endocrinological aspects of dysmatu-
- 13 MacRae D J, Willmott M P & Mohamedally S M. Clinical and endocrinological effects of intermittent abdominal decompression in the complications of pregnancy. *SA Med J* 46 1027 1972
- 14 Masson G M & Wilson G R. Variability of total plasma oestrol in late human pregnancy. *J Endoc* 54 245 1972
- 15 Roy E J. The concentrations of oestrogens in maternal and foetal blood obtained at Caesarean section and the effect of hospitalisation on maternal blood oestrogen levels. *J Obstet Gynaecol Br Commonw* 69 196 1962
- 16 Scommegna A & Chatteraj S C. Gas chromatographic estimations of urinary oestrogens in pregnancy. *Am J Obstet Gynecol* 99 1087 1967
- 17 Shearman R P, Jools M D & Smith I D. Maternal and fetal venous plasma steroids in relation to parturition. *Am J Obstet Gynecol* 79 717 1967
- 18 Taylor M F & Shackleton C H L. New oestrol in pregnancy urine. *Steroids* 24 185 1974
- 19 Taylor M F & Shackleton C H L. Identification and measurements of oestrols and oestrolones in pregnancy urine. *Research on Steroids* 7 497 1977
- 20 Varma T R & Curzen P. The effects of abdominal decompression on pregnancy complicated by the small-for-dates fetus. *J Obstet Gynaecol Br Commonw* 80 1086 1973
- 21 Wallace S J & Michie E A. A follow up study of infants born to mothers with low oestrol excretion during pregnancy. *Lancet* 2 1460 1966

Submitted for publication Aug 7 1977

M P Willmott
Northwick Park Hospital
Harrow Middlesex
England

PREOPERATIVE CERVICAL MICROBIAL FLORA AND POST ABORTION INFECTION

P J Moberg¹ P Eneroth² J Harlin³
Åsa Ljung³ and C E Nord⁴

From the ¹Department of Obstetrics and Gynecology the ²Hormone Laboratory of the Department of Obstetrics and Gynecology the ³Department of Clinical Microbiology Karolinska Sjukhuset Stockholm and the ⁴National Bacteriological Laboratory Stockholm Sweden

Abstract. With the aim to find criteria for the prediction of the patients who are at risk of developing a post abortion infection the pretreatment cervical microbial flora was compared between one series of patients who developed and another series of patients who did not develop such an infection. Aerobic and anaerobic bacteria as well as mycoplasma and fungi were studied in 104 patients. The distribution of aerobic and anaerobic bacteria was similar in the 14 patients who later developed post abortion infection and in uncomplicated cases. It is concluded that the distribution of the cervical microbial flora cannot serve as a basis for the prediction of which patients will develop subsequent genital infections.

One of the important complications of first trimester abortion by vacuum aspiration (VA) is pelvic infection (1 5 7 11 16). The incidence of this complication varies (1 5 16) widely (0.3–18%) due to differences in 1) definitions of post abortion infection 2) use of prophylactic antibiotic treatment and 3) time of observation.

Few reports on infection complicating VA have dealt with the factors which might have had an influence on the occurrence of pelvic infection after abortion (1 13). In some reports on the normal microbial flora of the cervix it has been pointed out that the cervix can be a reservoir of potentially pathogenic microorganisms (9 17) which have been regarded as normal contaminants. However, it has been postulated that these microorganisms do not cause symptoms under normal conditions (10 17). It has also been indicated that the microorganisms which predominate in female genital tract infections affect the normal vaginal flora. The cervix is believed to be the portal of entry (3 8 22).

In an attempt to predict those patients who would develop a post abortion infection a prospective study was performed. The cervical microbial flora—

including aerobic and anaerobic bacteria as well as mycoplasma and fungi—was studied immediately before the abortion. No prophylactic antibiotics were administered. The initial microbial flora of the patients who developed post abortion infection was compared to that of the uncomplicated cases. If the cervical microbial flora is an important factor in the subsequent development of infection after the abortion, it would be expected to be different in the two groups.

MATERIALS AND METHODS

The patients were 104 healthy women, aged 14–42 (Table I) who underwent abortion in the first trimester. The gestation was calculated from the first day of the last menstrual period (Table II). At a general examination 2–10 days prior to operation, none of the patients reported receiving antibiotic therapy during the previous month. No signs of genital infection requiring local therapy was revealed. Specimens for cultivation of *N. gonorrhoeae* were taken.

Operation was performed on an outpatient basis. Specimens for aerobic and anaerobic bacterial cultivation were taken by inserting dry charcoaled cotton swabs 10–15 mm into the cervix. One swab was put into Stuart's modified transport medium and brought to the Dept. of Clinical Microbiology, Karolinska Hospital. The second one was put into Shepards U9 broth with penicillin (18) and was kept at +4°C during the transportation to the Dept. of Clinical Microbiology, Uppsala, where cultivation and identification of *T. mycoplasma* was performed. The vagina was cleansed with 0.05% aqueous chlorhexidone (ACO Sweden). Paracervical block was followed by dilatation with Hegar dilators. After 5 IU of Oxytocin had been administered intravenously, vacuum aspiration was performed with a metal cannula. A check curettage with a blunt curette was carried out in all cases. No immediate postoperative complications occurred.

All patients were observed at the hospital for 5 hours following the operation. They were instructed in contact

Table I Distribution of patients according to age

Age	N
-14	1
15-19	19
20-24	16
25-29	37
30-34	70
35-39	8
40-	3
Total	104

the hospital in case of pelvic pain temperature rise to 38.0°C or above foul vaginal discharge or blood loss exceeding an ordinary menstruation. The observation time was two months.

The swab in Stuart's modified transport medium was cultured within two hours on the following media: one blood agar plate (Columbia agar BBL Cockeysville Md USA with 5% horse blood), one agar plate containing 3.55% phenylethanol agar (Difco Detroit Michigan USA), one Endo agar plate (Difco) all of which were incubated aerobically for 24 h at 37°C, one blood agar plate containing 0.01% gentian violet which was incubated anaerobically in a Gas Pak jar (BBL) for 24 h at 37°C, one haematin agar plate (BBL) which was incubated for 24 h in 10% carbon dioxide atmosphere at 37°C and one freshly prepared blood agar plate which was incubated anaerobically for 48 h at 37°C. This plate was kept for 5 days to allow appearance of *B. melaninogenicus*. Finally the swab was inserted in dextrose serum broth and thioglycollate broth and incubated for 24 h at 37°C before microscopic examination.

Identification of the aerobic and anaerobic bacteria was made according to Cowan & Steel and Holdeman & More (4, 12). Coagulase negative staphylococci were typed to the species level according to the scheme of Kloos & Schleifer (14). Coagulase positive staphylococci were phage typed (7). Typing of isolated fungi was performed at the National Bacteriological Laboratory.

RESULTS

Out of the 104 patients who had an abortion 89 had no signs of infection during the two months following the operation. Fifteen patients contacted the hospital because of discomforting gynecological symptoms. One of these patients had no objective symptoms and received no treatment. Seven of the patients were treated with antibiotics on an outpatient basis due to symptoms of a mild or suspected uterine and/or adnexal infection which made them seek advice at the hospital three days to six weeks after the abortion. Seven of the patients were hospitalized upon judgement by the gynecologist on duty because of an endometritis/salpingitis and

were treated with amoxycillin and/or doxycycline in adequate doses. In six of these patients the diagnosis was clear and was confirmed by a consultant gynecologist. The diagnosis of adnexal infection could not be confirmed in one of the hospitalized patients. Five of the hospitalized patients with an obvious gynecological infection were admitted within 48 hours after the operation. One patient with an obvious gynecological infection was hospitalized 15 days after the operation.

Relevant clinical data are presented in Table III.

The patients have been divided into three groups: 1) women with no complications, 2) women with mild or suspected genital infection (patients 1-7 and 3) women with clear genital infection (patients 8-14).

The initial cervical aerobic and facultative anaerobic bacterial flora as presented separately for each group of patients in Table IV. Seventeen patients had a positive fungal growth and 5 patients had a positive *Mycoplasma* culture. The findings were evenly scattered among the three groups. Most patients had a polymicrobial flora. The number of cultures with strictly anaerobic growth was similar in the three groups as seen in Table V.

DISCUSSION

The reported frequency of genital infections complicating first trimester abortions by VA varies widely (11). Benic et al (11) and Jurukovski & Sukarov (13) presented a low occurrence of post-abortion infections: 0.3% and 1.3% respectively. These authors reported regular prophylactic use of antibiotics or sulfonamides. Stallworthy et al (7) reported a 15% incidence of pyrexia 38°C or more for 24 hours or longer and an incidence of 3.4% for pelvic inflammatory disease/septicaemia/peritonitis. Moberg et al (16) reported a post-abo-

Table II Distribution of patients according to gestational age

Gestational week	N
7	4
8	0
9	39
10	30
11	8
12	3
Total	104

Table III Clinical data and microbial findings in patients with postabortal infection

* outpatient treatment H=hospitalization

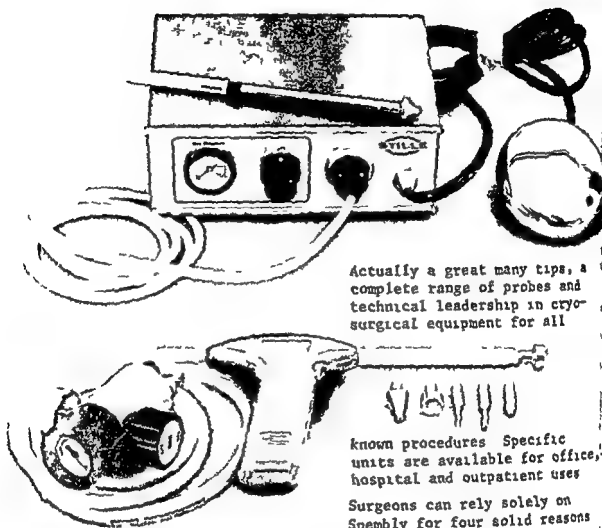
Case	Gest week	Interval abortion-admittence	Diagnosis	Treatment	Microbial findings in the cervix at the abortion
1(BL)	9	11 weeks	Salpingitis	Ampicillin OP	Strept intermed Eubact lentum Lact fermentum
1(LE)	10	3 days	Endometritis Salpingitis	Doxycycline Amoxicillin OP	Strept faecalis Torulopsis glabrata
1(LH)	8	15 days	Susp endometritis	Erythromycin OP	Bact pneumosintes Strept sanguis
1(EG)	8	3 days	Endometritis	Doxycycline Amoxicillin OP	Strept intermedius Strept mutior
1(K)	8	6 weeks	Susp endometritis	Erythromycin Doxycycline OP	Staph hominis Strept durans
1(B-C)	10	6 days	Endometritis	Doxycycline Amoxicillin OP	Lact buchneri
1(I)	9	15 days	Susp endometritis	Amoxicillin OP	No growth
1(B)	9	7 days	Endometritis	Amoxicillin H	Staph saprophyticus
1(Q)	10	2 days	Endometritis	Amoxicillin Doxycycline H	Strept sanguis
1(S)	7	2 days	Endometritis	Amoxicillin Doxycycline H	Staph haemolyticus Strept durans
1(L-13)	11	1 day	Endometritis Parametritis	Amoxicillin H	Lact fermentum
1(L-17)	9	1 day	Endometritis Parametritis	Amoxicillin Doxycycline H	Peptococcus magnus
1(G)	10	15 days	Endometritis Parametritis	Amoxicillin H	Lact buchneri
1(J)	9	6 days	Susp endometritis Parametritis	Amoxicillin H	Staph epidermidis Strept agalacticus Strept intermedius
1(N)	10	5 days	No infection	None	Bact pneumosintes

infection rate of 7.2% in 1123 patients in 1971. The figure included early as well as late abortion infections. Although the reported figures for infection vary due to a heterogeneous post-abortion infection concept based on both subjective symptoms and objective signs (16) the figures are in our opinion unacceptably high. The aim of the present study was to select the group of patients at risk of developing a post-

abortion infection. If the occurrence of a postabortal infection was clearly related to microbial pathogens present in the cervix it should be possible to identify these patients prior to the operation. In several reports of female upper genital tract infections anaerobic bacteria are the predominating organisms in a polymicrobial flora (3-21).

The normal flora of the cervix is also a complex polymicrobial composition (9-11) and

Spemby has a little tip for all cryosurgeons



Actually a great many tips, a complete range of probes and technical leadership in cryosurgical equipment for all

known procedures Specific units are available for office, hospital and outpatient uses

Surgeons can rely solely on Spemby for four solid reasons

- excellence of equipment performance and design
- world wide experience and distributor network
- comprehensive technical and instructive literature
- and the knowledge that Spemby is continually developing cryosurgical applications and techniques

If you would like more information and literature on the Spemby range please contact your local Spemby supplier or write to us in Andover Spemby Limited, Newbury Road, Andover, Hampshire SP10 4DR, England

Telephone (0264)65741 Telex 47403

Or to **STILLE**

SWEDEN AB STILLE-WERNER, Box 43051, S-100 72 Stockholm

FINLAND OY STILLE AB, Nervanderinkatu 5 D,

SF-00100 Helsinki 10

NORWAY STILLE A S, Postboks 61 Leirdal, Oslo 10

SWITZERLAND STILLE AG, Postfach, CH-8038 Zurich

Spemby

FIRST CHOICE
IN CRYOSURGERY

TREATMENT OF PRIMARY DYSMENORRHEA WITH PROSTAGLANDIN SYNTHETASE INHIBITORS—A PROMISING THERAPEUTIC ALTERNATIVE

Viveca Lundstrom

*From the Department of Obstetrics and Gynecology
Karolinska Institute Stockholm Sweden*

Abstract Three groups of patients with primary dysmenorrhea were treated with prostaglandin synthetase inhibitors. Thirty-one women received indomethacin at a dose of 50 mg \times 3-4 per day usually starting one to two days before the onset of menstruation and 38 women received naproxen 50 mg \times 3-4 per day usually starting on the first day of bleeding (open studies). Seventy-one percent of the patients experienced moderate or good relief of pain following indomethacin and 67% following naproxen. In a third series a double blind crossover study using the sodium salt of naproxen versus placebo in 26 patients showed that naproxen sodium was significantly more effective than the placebo ($p < 0.05$). At the doses employed the prostaglandin synthetase inhibitors were not associated with any side effects of major concern. The study indicates that this form of therapy offers an effective alternative in patients who for some reasons do not accept hormonal treatment.

Dysmenorrhea represents one of the commonest complaints in gynecological office practice. The social handicap that this disorder imposes upon young working women, students and professionals is considerable. Combined oral contraceptives or dehydro-epiandrosterone offer efficient relief in a number of cases. There is, however, a significant segment of the female population who neither accept nor tolerate steroid medication. The value of narcotic analgesics and/or sedatives is limited and these agents are potentially dangerous. A therapeutic alternative is urgently needed. Prostaglandin (PG) synthetase inhibitors have proved to be successful in many cases of incapacitating dysmenorrhea but so far few well controlled studies are available on their clinical usefulness in this condition.

In this series of clinical trials we investigated the effect of prostaglandin synthetase inhibitors in the treatment of dysmenorrhea. We proceeded in several steps. First, in an open trial we investigated the

therapeutic efficacy of a traditional PG synthetase inhibitor—indomethacin; thereafter we used a novel compound with prostaglandin inhibitory properties—naproxen (20) again in an open trial. Subsequently, of the strong placebo effects of any medication in dysmenorrhea we designed a double blind crossover trial using the sodium salt of naproxen (naproxen Na).

Several other aspects of the therapeutic efficacy of PG synthetase inhibitors were studied. Firstly, we wanted to learn whether starting the treatment several days before the onset of bleeding would eliminate the pain more effectively than starting the treatment with the beginning of the menstrual flow. This question arose in association with studies demonstrating that indomethacin decreased urinary prostaglandin metabolites by 85% after two days of administration (9). Secondly, a causative relationship has been suggested between gastrointestinal symptoms of dysmenorrhea and high concentrations of circulating endogenous PGs; we questioned therefore whether dysmenorrheic patients suffering from such symptoms may be particularly suited for treatment with PG synthetase inhibitors. Another clinically important problem was whether dysmenorrhea in women who did not obtain relief with the administration of combined oral contraceptives would respond to PG synthetase inhibitors.

Finally, we investigated the effects of naproxen Na on the uterine contractions and tried to define the relationship between pain relief and the decrease of uterine tone.

MATERIAL AND METHODS

Selection of patients

Women suffering from dysmenorrhea were referred to the outpatient clinic of the Department of

Karolinska Hospital and to a community hospital in the vicinity of Stockholm. There they were interviewed and examined by the investigator.

Prior to admission to the study a thorough history of the menstrual distress was obtained apart from uterine cramps other symptoms associated with dysmenorrhea were recorded such as nausea vomiting diarrhea headache fever and shivering. The pain relief obtained with combined oral contraceptives or analgesics was also noted. Thereafter the subjects received a complete pelvic examination. Only women with normal gynecological findings and who required analgesics regularly (moderate dysmenorrhea=degree 2) or who had to refrain from work (severe dysmenorrhea=degree 3) were selected for the study. Women with dysmenorrhea associated with other pathological disorders such as chronic salpingitis or endometriosis were excluded from the trial. One patient who had a bicornuate uterus was accepted. Patients who expressed a desire for oral contraceptives were excluded. No attempt was made to evaluate possible psychological factors associated with dysmenorrhea.

Study groups and treatment schedules

Three main groups and one additional special group of patients constituted the trial.

Group 1 included 31 patients treated with indomethacin (Indomec® Merck Sharpe and Dome) during two cycles. Dysmenorrhea in 13 patients was classified as moderate (degree 2) and in 18 as severe (degree 3). Fourteen patients indicated gastrointestinal symptoms associated with menstrual cramping.

The patients were recommended to take indomethacin in an oral dose of 25 mg three or four times daily starting one or two days before the expected menstruation and to continue until the pain normally would have disappeared. In case a pregnancy was planned patients were recommended not to start the treatment until the beginning of the menstrual flow. Twenty-two patients started the treatment before the onset of the bleeding and nine on the first day of bleeding.

Group 2 Thirty-nine patients were selected for the treatment with naproxen (Naprosyn® naproxen (d 2 (6-methoxy 2 naphthyl propionic acid) and naproxen sodium kindly supplied by Syntex Research) during three cycles. Dysmenorrhea in nine patients was classified as moderate (degree 2) and in 30 as severe (degree 3). A history of gastrointestinal symptoms associated with dysmenorrhea was indicated by 18 patients.

The patients were recommended to take naproxen in a dose of 250 mg twice daily starting one to two days before the expected menstruation and increase the dosage to 750 mg three to four times daily during the days of the actual menstrual flow. If the treatment proved ineffective when starting one to two days before menstruation patients were recommended to start four to five days before the expected bleeding. Eleven patients succeeded in starting the medication before the onset of bleeding while 28 started during the first day of the menstrual flow.

Group 3 Twenty-eight patients were selected for the double blind crossover study with naproxen sodium versus placebo. Dysmenorrhea in 20 patients was classified as severe (degree 3) and in 8 as moderate (degree 2).

Twenty patients indicated a history of premenstrual symptoms associated with dysmenorrhea.

On admission to the trial the patients were asked by the investigator to compare two analgesics for menstrual pain during a six month period using each compound successively over a three month period. The patients were instructed to start the study medication on the first day of menstruation. Naproxen Na was to be taken in a dose 550 mg (7 tablets each 275 mg) followed by 775 mg every six hours to a maximum daily dose of 1100 mg. The same number of tablets with identical physical properties was prescribed for the placebo treatment. The assignment of patients to the naproxen Na or to the placebo treatment and the sequence in which patients received the six drugs proceeded according to a randomization schedule. For each treatment month on special forms the patient indicated the degree of menstrual pain gastrointestinal symptoms the amount of bleeding effect of the treatment and side effects. After three months the patients were interviewed by the investigator and medication was handed out to them for another three month period.

Moreover in this group hemoglobin concentration leucocyte and thrombocyte counts as well as hepatic function tests were analyzed before the start of medication and after three and six months of treatment.

The patient characteristics of the three treatment groups are summarized in Table 1 indicating the number of patients their age parity and character of dysmenorrhea (historical presence of gastrointestinal discomfort family incidence of dysmenorrhea and the degree of dysmenorrheic pain).

The trials included an additional special group consisting of three patients with dysmenorrhea and two non-dysmenorrheic women. In these women the uterine contractility was investigated after a single oral dose of 54 mg of naproxen Na. Uterine contractions were recorded by means of an immobilized micro-balloon which was introduced into the uterine cavity and connected through a pressure sensor to a recording device (13).

Evaluation of treatment effects

After each treatment course the patients completed special forms indicating the degree of menstrual pain the intensity of bleeding the presence of gastrointestinal symptoms the relief achieved by the study drug and side effects. At an interview the investigator evaluated each patient's regularity of drug intake the changes in dysmenorrheic symptomatology and in the menstrual flow pattern.

The relief achieved by the study medication was scored according to the following system:

- 0 No improvement
- 1 Moderate improvement (still pain but less severe than normally)
- 2 Marked improvement (slight pain present occasionally)
- 3 Complete relief

In the first overall analysis the effectiveness of the study drug was assessed by the percentage of women achieving marked improvement (scores 2 to 3) and moderate improvement (score 1) as compared with the percentage of

Table 1 Characteristics of patients in the different treatment groups

Treatment	No. of patients	Mean years of age \pm S.D.	Nullipara	Multipara	Gastro-intestinal discomfort	Family incidence of dysmenorrhea	Degree of dysmenorrhoeic pain*	
							2	3
Indomethacin	31	27.9 \pm 7.0 (16-41)	24	7	14	15	13	18
Naproxen	39	23.4 \pm 8.0 (13-47)	37	7	18	17	11	30
Naproxen sodium	8	24.7 \pm 8.0 (16-47)	26	-	11	16	6	20
Placebo	58		8	16	50	48	28	68

*Dysmenorrhea present in mothers and/or sisters of patients

2=severe dysmenorrhea i.e. requiring regular use of analgesics 3=severe dysmenorrhea i.e. causing loss of working or school days

those whose dysmenorrhoeic pain remained unchanged in the analysis of the double blind crossover study (group 1b). Scores achieved during individual treatment courses were summed for the three months placebo period. The statistical methods employed in analysis are described in the Results section below.

In the special group where uterine contractions were recorded the time to reduce uterine activity and its maximum reduction following a dose of 550 mg of naproxen were recorded and correlated with pain relief times.

RESULTS

Analysis of the different treatment groups

The overall results of the three groups studied are presented in Table II with pain relief, side effects and changes in menstrual pattern recorded numerically.

Group 1 Indomethacin

Among the 31 patients who were treated with indomethacin there was complete relief or marked decrease in pain in 20 (65%) and a moderate effect in two (6%). The treatment was ineffective in 29%. Four patients reported side effects probably induced by the drug, namely four cases of headache, one of exanthema, one of nausea and one of severe rashes which forced the patient to stop the treatment immediately. Decreased bleeding was observed by two patients, increased bleeding by one and there was a delay of menses of 5-7 days in two subjects.

Group 2 Naproxen

Of cases treated with naproxen good pain relief experienced by 23 subjects (59%) while three had moderate relief. No improvement was found in 33%. Side effects probably due to medica-

tion occurred in three patients: headache in two cases and fatigue in one. Decreased or shortened menstrual flow was observed by ten subjects, increased bleeding by two and delay of menstruation by a further two.

Group 3 The double blind crossover trial with naproxen Na

(a) The overall effect of the study drugs: Twenty eight patients started the first three month period of the study, 14 on naproxen Na and 14 on placebo. However, only 26 patients (two patients became pregnant after the first three months of treatment) crossed over and started the second three month period (13 on naproxen Na and 13 on placebo). As shown in Table II, naproxen Na: 22 patients (85%) experienced marked or moderate improvement and four (15%) no improvement. The corresponding figures for the placebo treatment period were 9 patients with marked to moderate improvement (34%) and 17 patients no improvement (65%). There was no significant difference between the two groups with regard to the incidence of side effects: in one patient the rash following naproxen Na could have been ascribed to the compound.

Menstrual disorders were recorded in eight cases of the naproxen Na group but in only four patients of the placebo group. In one patient of the former group the duration of menstrual flow decreased from six days to only one day and this was accompanied by complete relief of the pain of her otherwise incapacitating dysmenorrhea.

(b) The crossover analysis: The effect of naproxen Na in the treatment of dysmenorrhea compared with a placebo is illustrated in Fig. 1. Each patient is presented individually and irrespective of the treatment order. The effective

Table 11 Pain relieving effect side effects and change in menstrual bleeding in the different treatment groups

Treatment	No of pts	Marked improvement (scores 2-3)		Moderate improvement (score 1)		No improvement		Side effects	
		No	%	No	%	No	%	No	%
Indomethacin 25 mg x 3-4	31	20	65	7	6	9	29	7	23
Naproxen 750 mg x 2-4	39	23	59	3	8	13	33	3*	8
Naproxen sodium 550 mg + 275 mg + 275 mg	26	13	50	9	35	4	15	4	15
Placebo	76	4	15	5	19	17	65	44	19

Indomethacin headache 4 gastritis 1 nausea 1 exanthema 1

* Naproxen headache 2 fatigue 1

Naproxen sodium headache 2 fatigue 1 exanthema 1

Placebo depression 2 headache 1 fatigue 1 dizziness 1

treatment was evaluated once a month and the horizontal bars represent the sum of the scores obtained during each three month period either on naproxen Na or placebo. Individual exceptions aside it seems evident that the scores for naproxen Na treatment were superior to those of placebo. Only four patients evaluated the placebo tablets higher than the active drug whereas twenty preferred naproxen Na.

The statistical method employed in the crossover analysis was the Wilcoxon signed rank test. Patients who completed only one treatment course on placebo or on naproxen Na (in Fig. 1 pointed out by asterisks) were excluded from analysis. Thus 22 patients could be evaluated: 10 in the naproxen Na→placebo sequence and 12 in the placebo→naproxen Na sequence. The analysis of the individual differences in scores between the naproxen Na period and the placebo period revealed a significant difference in favour of naproxen Na with a *p* value of 0.015.

(c) *Parallel analysis* Parallel analysis for the first three month period only was performed as well. The therapeutic effects of naproxen Na in 14 patients was compared with the effect achieved by placebo in the other 14. The Wilcoxon two sample test yielded a *p* value of 0.024 in favour of naproxen Na.

(d) *Laboratory tests* No abnormal changes in hemoglobin, leucocyte count and hepatic function tests were observed after treatment with either naproxen Na or placebo. One patient treated with naproxen Na showed a drop in thrombocyte count from 250 to 100 thousand/mm³. After two cycles the values were 222 again. However, a similar drop was

also observed in three placebo cases: 216 to 190, 315 to 160 and 200 to 135. These may indicate spontaneous variations or erroneous counting by the routine laboratory.

Initiation of therapy in relation to the commencement of menstrual flow

Table III represents an analysis of the pain relieving effect of indomethacin and naproxen with regard to the day of the cycle on which the treatment was started.

Patients who obtained PG synthetase inhibitors 1-5 days before the onset of their menstrual flow were compared with those treated from the first day of bleeding. The overall results indicate that there was no difference in pain relief between these two modes of administration.

However, the present case material also included exceptional cases where the duration of PG synthetase inhibitor treatment premenstrually did seem to be of importance for the alleviation of dysmenorrheic pain.

One patient, a 17 year old nulligravida with dysmenorrhea since the age of 12, had suffered from severe pain and frequent vomiting to the extent that she had to stay in bed for one to two days every month. She did not experience any relief with indomethacin 50 mg rectally twice a day one day prior to menstruation nor with naproxen orally at a dose of 250 mg three times daily starting two days before expected menstruation. However, when naproxen medication was begun 5-6 days before menses there was complete relief of pain as well as of all her gastrointestinal symptoms. This effect was observed during three different cycles.

2nd cycle	Decreased or shortened bleeding		Delay of menses	
	No	%	No	%
1	7	6	2	6
2	10	6	2	5
3	11	23	3	4
4	3	17	0	-

Gastrointestinal symptoms

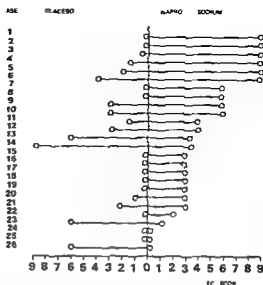
Nausea, vomiting and diarrhoea occurred in about 50% of the dysmenorrhoeic women. It was interesting to note that women who experienced a significant relief of uterine pain by PG synthetase inhibitors also experienced a disappearance of their gastrointestinal disturbances. Table IV represents a separate analysis of patients with and without gastrointestinal symptoms (vomiting and/or diarrhoea) accompanying the period of menstrual pain. However, the pain relieving effect of PG synthetase inhibitors did not differ significantly between these categories of patients.

Oral contraceptives

Seven women suffered from dysmenorrhoea in spite of the fact that they had been using combined oral contraceptives. These women represent a cross section from all three groups. When treated with PG synthetase inhibitors eight experienced complete relief or marked improvement of their symptoms.

The effect of naproxen Na on uterine contractility

As illustrated in Fig. 2 naproxen Na exerted a relatively prompt effect on uterine contractility in the whole group of five patients. The spastic and hyperemic contractility pattern of the uterus in cases of dysmenorrhoea was altered within a period of approximately one hour to attain contractions of significantly lower amplitudes, lower frequency and slower basal tone. This change in contractility was followed by complete disappearance of pain in all five dysmenorrhoeic women in whom the uterine activity was registered. This suggested a very rapid



* COMPLETE RELIEF ON ONE CYCLE ON PLACEBO

* COMPLETE RELIEF ON ONE CYCLE ON NAPROXEN SODIUM

Fig. 1 Diagram illustrating the results of a double blind crossover study of naproxen sodium versus placebo in the treatment of dysmenorrhea. The horizontal bars on each side of the vertical line represent the sum of pain relief scores during three cycles.

action of the orally administered naproxen Na. A similarly rapid change in uterine contractility was also observed in the two women not suffering from dysmenorrhoea.

DISCUSSION

The first report on the use of anti-inflammatory drugs for the treatment of dysmenorrhoea appeared in 1953. Fox (7) observed that women treated with phenylbutazone for rheumatic conditions experienced a decrease in their menstrual pain and discomfort. Twenty years later Hill (11) and Christensen (3) reported that indomethacin had a favourable analgesic effect in women with dysmenorrhoea. Apparently none of these authors were aware of the probable mechanism of action of such anti-inflammatory drugs, namely that of the inhibition of prostaglandin synthesis. Layes Molla & Donald (16) who compared the effect of ibuprofen and paracetamol in a double blind study of dysmenorrhoeic women might not have recognized the significance of this mechanism since neither prostaglandins nor prostaglandin synthetase inhibitors were mentioned in their paper. The reported complete relief or improvement of symptoms in 62-64% of their cases in the two groups

Table III *Pain relieving effect of indomethacin or naproxen when starting medication before or at the beginning of menstruation*

Groups I and II	No of pats	Marked improvement		Moderate improvement		No improvement	
		No	%	No	%	No	%
Patients starting medication before onset of menses	33	20	61	2	6	11	31
Patients starting medication at the beginning of menses	37	26	70	2	5	9	24

Vane's (1971) experimental studies on the mechanism of action of aspirin and indomethacin created a sound basis for the understanding of the beneficial effects of these drugs on dysmenorrhea (21). With this discovery in mind Schwartz et al (19) conducted the first clinical study of the effect of flufenamic acid in cases of dysmenorrhea. The trial comprised 16 patients who took flufenamic acid orally during a total of 31 cycles. The drug abolished the symptoms in all patients. The same group of women obtained analgesics, spasmolytics, tranquilizers or placebo over 72 cycles but these afforded no significant relief. Similarly Massey et al postulated that the analgesic effect of naproxen in uterine pain following IUD insertion was due to the prostaglandin inhibitory effect of this compound (16).

The correlation between high uterine tone and dysmenorrheic pain has been suggested by several authors (1, 4, 5, 6). In an earlier study Lundstrom et al (13) established that oral and rectal administration of indomethacin decreased the uterine tone as well as the amplitude and frequency of the contractions in dysmenorrheic women and that these alterations in uterine activity occurred simultaneously with the relief of pain.

Csapo et al (5) and Lundstrom & Gr  n (14) made the same observations using naproxen Na.

The latter authors also showed that at the beginning of menstruation the concentration of $PGF_{2\alpha}$ as determined by gas chromatography-mass spectrometry was higher in the endometrium of dysmenorrheic women than in non-dysmenorrheic subjects.

Boehm & Sarratt (2) reported the results of an open clinical study where 83% of 30 women experienced pain relief by taking indomethacin (40 mg, three times a day). A clinical double blind study of the effect of PG synthetase inhibitors in the treatment of dysmenorrhea by Henzl et al (17) showed that naproxen Na had a significant pain relieving effect.

The case material in the present study was divided into three groups of which groups I and II may be characterized as common open studies. Seventy one per cent of the patients in the indomethacin series and 67% of the naproxen treated patients obtained complete or partial relief from their pain. These results are approximately the same as those achieved by other investigators.

Dysmenorrhea is a multifactorial syndrome where psychological influences may play a role. Moreover, cycle irregularities as well as the difficulty of measuring pain and the degree of pain relief create problems in the objective evaluation of the results. A carefully controlled double blind study is

Table IV *Correlation between gastrointestinal symptoms and pain relieving effect*

Condition	Number	Marked improvement		Moderate improvement		No improvement	
		No	%	No	%	No	%
Dysmenorrhea with gastrointestinal symptoms	50	31	62	7	14	1	2
Dysmenorrhea without gastro- intestinal symptoms	46	29	63	33	72	15	33

Apur. m. mod. m. 550 mg

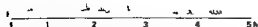


Fig. 2 Recording of uterine contractility in a woman suffering from severe menstrual pain (day II of menses). One hour after oral administration of naproxen sodium reduction of uterine tonus and frequency and amplitude of contractions is seen. Simultaneously with the decrease in uterine contractility the patient became completely free from symptoms.

Therefore of major importance for an evaluation of drug effect.

Although it is difficult to complete a double blind crossover study in dysmenorrheic women it is believed that this design offers a more accurate assessment of the results than simple double blind trials since the same patient experiences the effect of both the active drug and placebo.

Group 3 in the present investigation participated in a double blind crossover study. The results showed that the symptoms of 85% of the volunteers in the naproxen Na group were completely or partially improved compared with 34% of the placebo group. The PG synthetase inhibitor effect is similar to that found by most other authors while the placebo effect corresponds to expected levels.

If the degree of pain is difficult to assess it is even more difficult to define and evaluate the occurrence of different drug induced side effects. This is illustrated by the fact that the placebo group had a higher incidence of side effects than the active drug groups of both groups 2 and 3. Although the indomethacin group had the highest overall rate of side effects of all three groups definite conclusions in this respect cannot be made. However the only side effect that occurred in the series was an instance of marked gastritis and this case belonged to the indomethacin group. Boehm & Sarraff reported a comparatively high incidence of side effects with indomethacin but they used twice the dose employed in this study. Differences between the groups with regard to changes in menstrual flow were difficult to assess. It should however be emphasized that there was a tendency towards a decrease in the duration of menstruation under PG synthetase inhibitor treatment.

An observation that may be of some clinical interest is that patients with both dysmenorrhea and gastrointestinal symptoms, namely diarrhea and vomiting (which are well known side effects of PG

administration) responded no more favourably than those without gastrointestinal symptoms. Analysis of the timing of medication indicated that the pain relieving effect was the same whether medication was started before expected menstruation or on the first day of menstrual bleeding. Thus the fact that most patients do not have to start taking PG synthetase inhibitors until the onset of symptoms of impending menstruation has obvious practical advantage. The rapid absorption of naproxen Na may be of particular significance when considering this aspect of administration. In some individuals however the administration of PG synthetase inhibitors prior to menstruation is necessary and clinically beneficial.

β_2 adrenergic stimulating drugs have recently been shown to inhibit uterine hypercontractility associated with dysmenorrhea (1). However these compounds do not appear to be clinically useful. Terbutaline taken orally reduced menstrual pain but caused too many side effects such as tremor and tachycardia to be acceptable (1). Other studies by Hansen & Secher (10) using hydroxyphenyl orciprenaline and by Nesheim & Walloe (17) using isoxuprine showed no significant effect in the relief of dysmenorrhea when compared with a placebo.

The most effective therapy for dysmenorrhea is still hormonal treatment. Combined oral contraceptives which reduce pain in 95 to 98% of the patients cause anovulatory cycles. Dehydro-retroprogesterone which does not block ovulation also offers very effective pain relief in this syndrome. The action of the latter compound is not clear. Pickles has demonstrated in one patient that an anovulatory cycle resulted in a decrease of PGF_2 in the menstrual fluid to a fifth of the content in an ovulatory cycle (18). Lundstrom (14) has shown that oral contraceptives reduced the PGF_2 concentration by 75% in the endometrium in one patient. Whether dehydro-retro-progesterone also reduces PG synthesis has not yet been studied but a similar mechanism seems possible.

CONCLUSION

Naproxen Na with its potent action on PG synthesis and rapid oral resorption combined with few side effects offers an effective therapeutic alternative in patients who do not accept hormonal therapy for the treatment of their dysmenorrhea or who do

not desire a birth control method anyway or who suffer from dysmenorrhea in spite of hormonal contraception

ACKNOWLEDGEMENT

The author is indebted to Astra Syntex Inc. Södertälje, Sweden, for the generous supply of the naproxen sodium and placebo tablets, to Dr Jerry Johnson, Syntex Research, Palo Alto, California, for the statistical evaluation of the results, and to Ms L. M. Lindeholm for excellent secretarial assistance. My thanks are due to Professor Nils Wijkvist and Dr Milan Henzl for valuable suggestions and a careful critical review of the manuscript.

The investigation was supported by the WHO Expanded Programme of Research, Development and Research Training.

REFERENCES

- 1 Åkerlund M, Andersson K E & Ingmarsson I. Effects of terbutaline on myometrial activity, endometrial flow and lower abdominal pain in women with primary dysmenorrhea. *Br J Obstet Gynecol* 83: 673, 1976.
- 2 Boehm F H & Sarrazin H. Indomethacin for the treatment of dysmenorrhea. *J Reprod Med* 15: 111, 1975.
- 3 Christensen A. Indomethacin mot mähimuna menstruala. *Ugeskr Laeger* 136: 592, 1974.
- 4 Csapo A I. The diagnostic significance of the intrauterine pressure. *Obstet Gynecol Surv* 25: 403, 1970.
- 5 Csapo A I, Pulkkinen M O & Henzl M R. The effect of naproxen sodium on the intrauterine pressure and menstrual pain of dysmenorrhoeic patients. *Prostaglandins* 13: 193, 1977.
- 6 Filler W & Hall J. Dysmenorrhea and its therapy. A uterine contractility study. *Am J Obstet Gynecol* 106: 104, 1970.
- 7 Fox W W. Butazolidine. Letter to the editor. *Lancet* 1: 195, 1953.
- 8 Halbert D H, Demers L M, Fontana J & Jones D E D. Prostaglandin levels in endometrial jet wash specimens in patients with dysmenorrhea before and after indomethacin therapy. *Prostaglandins* 10: 1047, 1975.

- 9 Hamberg M. Inhibition of prostaglandin synthesis by aspirin. *Biochem Biophys Res Commun* 49: 770, 1977.
- 10 Hansen M K & Secher N J. Beta receptor stimulation in essential dysmenorrhea. *Am J Obstet Gynecol* 121: 566, 1975.
- 11 Hill G G. Is your pain really necessary. Correspondence. *Br Med J* 106: 1973.
- 12 Henzl M R, Buttram V, Segre E J & Bessler S. The treatment of primary dysmenorrhea with naproxen sodium. A report on two independent double blind trials. *Am J Obstet Gynecol* (in press).
- 13 Lundström V, Green K & Wijkvist N. Prostaglandins, indomethacin and dysmenorrhea. *Prostaglandins* 11: 893, 1976.
- 14 Lundström V & Green K. Endogenous levels of PGF_2 and its main metabolites in plasma and in endometrium of normal and dysmenorrhoeic women. *Am J Obstet Gynecol* 130: 640, 1978.
- 15 Layes Molla A & Donald J F. A comparative study of ibuprofen and paracetamol in primary dysmenorrhea. *J Int Med Res* 2: 395, 1974.
- 16 Massey S E, Varady J C & Henzl M R. Pain relief with naproxen following insertion of an intrauterine device. *J Reprod Med* 13: 226, 1974.
- 17 Nesheim B I & Walloe L. The use of isoxuprine in essential dysmenorrhea. *Acta Obstet Gynecol Scand* 53: 315, 1976.
- 18 Pickles V M. Prostaglandins in the human endometrium. *J Fertil* 12: 335, 1967.
- 19 Schwartz A, Zor W, Lindner H R & Naor S. Primary dysmenorrhea. Alleviation by an inhibitor of prostaglandin synthesis and action. *Obstet Gynecol* 44: 709, 1974.
- 20 Tomlinson R V, Ringold H J, Qurechi M C & Forchielli R. Relationship between inhibition of prostaglandin synthesis and drug efficacy: support for the current theory on mode of action of aspirin-like drugs. *Biochem Biophys Res Commun* 46: 451, 1972.
- 21 Vane J R. Inhibition of prostaglandin synthesis: the mechanism of action for aspirin-like drugs. *Nature* 231: 232, 1971.

Submitted for publication May 2, 1977

Viveca Lundström
Dept of Obstetrics and Gynecology
Karolinska Sjukhuset
S-10401 Stockholm
Sweden

RECORDING OF MYOMETRIAL ACTIVITY IN THE NON PREGNANT HUMAN UTERUS BY A MICRO TRANSDUCER CATHETER

M Åkerlund L Ph Bengtsson and U Ulmsten

From the Departments of Obstetrics and Gynaecology Lund and Malmö
University of Lund Sweden

Abstract A micro-transducer catheter was tested *in vitro* and *in vivo* for recording of myometrial activity in the non-pregnant human uterus and was also compared with conventional fluid filled open end catheters previously used for recording intra uterine pressures. The micro-transducer catheter had a frequency response far above that of fluid filled open end catheters. It was easily introduced through the uterine cervix and was never obstructed. Provided that the sensory surface of the micro-transducer was kept from direct contact with the uterine wall by some special arrangement it gave recordings of the intra-uterine pressure that were practically identical with those obtained by an open end catheter.

A variety of catheters have been used for recording intra uterine pressure in non pregnant women. Braksma et al (6) made a comparison both *in vivo* and *in vitro* and found that open end (7) and spongetipped (4) catheters gave reliable results whereas closed tipped catheter systems (membrane micro-balloon or ballontip catheters) did not.

However the open end catheter often becomes obstructed at the intra uterine end by mucus blood or endometrial fragments and requires therefore repeated flushing or continuous counter pressure. The spongetipped catheter also sometimes becomes obstructed but less frequently than the open end catheter. Furthermore the occurrence of leakage or air bubbles in the fluid filled open end catheter may result in distorted recordings.

All these functional disadvantages of fluid filled catheters might be overcome by the recently constructed micro-transducer catheter. Such a catheter has until now mainly been used for recording pressure in body cavities such as the heart the large blood vessels and the female urinary tract (3, 8, 10, 11).

The aim of the present study was to investigate

whether such a micro-transducer catheter could be used for recording myometrial activity in the non pregnant uterus. In the same study we also wished to compare the micro-transducer catheter with conventional fluidfilled open tip catheters previously used for recording intra uterine pressure. For this comparison we chose the open end catheter in order to keep the added volume of the intra uterine part as small as possible.

MATERIAL AND METHODS

Recording systems

The construction of the two different recording systems is briefly as follows:

The micro-transducer catheter consists of a dacron catheter 2.31 mm outer diameter. In the tip of this catheter a micro-transducer is enclosed (Millar Instr. Houston Texas USA). The sensor is a silicon semiconductor unbonded strain gauge with the sensor surface at the side of the catheter tip. According to the manufacturer it has the following technical data: Frequency response 0 (dc)–20 kHz. Internal calibration 100 mmHg \pm 2 mmHg. Range –300 to +400 mmHg. Reference pressure Vent to atmosphere. Hysteresis and linearity Error less than 0.5% F.S.

These data were confirmed by tests in the department of bio-engineering at our hospital. The thermal stability given in the manufacturer's manual (\pm 0.1 mmHg/°C at 25–40°C) did not always prove to be reliable in our tests and the experiments and calibrations were therefore all ways done at a constant temperature (37°C).

The pressure transducer was connected to an amplifier (EMT 311 Elema Siemens Stockholm Sweden) and a potentiometer recorder (Omniscrite Houston Instr. Texas USA).

The open end catheter recording system consists of a catheter made of vinyl the inner diameter 0.5 mm and the outer diameter 0.9 mm. The length of the catheter is 1 meter. The outer end of the catheter was attached to a conventional pressure transducer (EMT 35 Elema Siemens) an amplifier (EMT 311 Elema Siemens) and the potentiometer recorder.

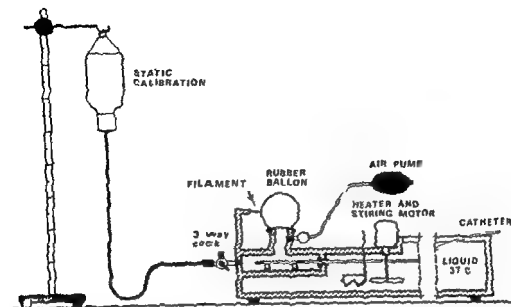


Fig 1 Cross section of the calibrating system ready for a dynamic calibration of the micro-transducer catheter which is inserted into the pressure chamber. The dynamic calibration is performed by bursting the rubber balloon connected to the fluid filled pressure chamber. The pres-

sure within the chamber is thereby momentarily reduced to atmospheric pressure. Another catheter can be introduced into the calibrator if the static calibration equipment is dismantled.

Experimental procedures

Studies in vitro Both recording catheters were calibrated and tested against each other in a specially designed test chamber where the pressure was regulated by a rubber balloon (Fig 1).

This test chamber or calibrator in which it is also possible to disinfect the catheters has previously been fully described (7).

Studies in vivo Twenty healthy women of fertile age who had been fully informed and had consented to the investigation were recorded in different periods of the menstrual cycle. In the first series of investigations the micro-transducer catheter was inserted alone into the uterine cavity so far that the pressure sensing section of the catheter was placed in the fundus.

In order to expose the sensory surface alternatively to

the anterior and the posterior walls and to the myos filled sh. between the walls the catheter was turned around its axes at a constant intra uterine level.

In the second series of investigations the microtransducer catheter was inserted tied to an open end catheter and a thermistor catheter (Fig 2).

This catheter has previously been used for simultaneous pressure and blood flow recording (1). As can be seen in Fig 2 the two additional catheters created a bridge over the sensory area of the micro-transducer catheter submitting it to the same pressure within the surrounding mucosa as the open end catheter. By this arrangement direct pressure of the uterine walls on the sensory area of the micro-transducer could be avoided.

RESULTS

The results from the *in vitro* investigations are presented in Figs 1 and 4.

From Fig 3 it is seen that the reaction time of the micro transducer catheter is <1 msec and that of the open end catheter about 80 msec. The results from the dynamic tests in the test chamber are illustrated in Fig 4. As can be seen the micro-transducer and the open end catheter recordings are in all respects identical sharing the same amplitude and shape of the recorded pressure change.

During the *in vivo* investigations it was found that

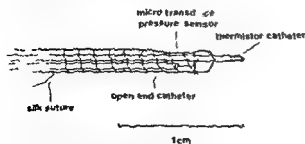


Fig 2 The pressure sensing section of a microtransducer catheter combined with a thermistor catheter and an open end catheter as they were used in the *in vivo* recordings.

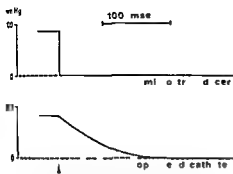


Fig 3 Reaction times of the two different recording systems tested in the calibrator. At the arrow the rubber balloon is burst.

The micro-transducer catheter caused almost no discomfort when introduced through the uterine cervix. It functioned without interruption during all the recordings even when they lasted up to 10 hours. The open end catheter on the other hand had to be flushed intermittently to function adequately. When the micro-transducer catheter was used alone *in vivo* it gave in different periods of the menstrual cycle patterns of pressure changes similar to those obtained previously by open end and sponge tipped catheters. However it was noticed that both amplitude and basal tonus in the recordings could sometimes be changed up to 10 mmHg when the direction of the sensory surface of the catheter tip in the uterine cavity was changed by turning the catheter around its axes. When the sensory area was kept from direct contact with the uterine walls by the thermistor catheter, turning of the micro-transducer catheter did not have any influence. Practically identical recordings were obtained from both the micro-transducer and the open end catheter (Fig 5).

DISCUSSION

The pregnant uterus contains a fluid filled cavity. In all parts of this cavity the pressure under static conditions is the same if differences due to hydrostatic pressure are overlooked. It is important to stress that the normal non pregnant uterus has no cavity but only a slit between the anterior and posterior wall filled by endometrium and a small amount of mucus. This has been fully proved by experiments where 2-3 small balloon catheters (9)

or 2-3 sponge tipped catheters (5) have been inserted into the uterine cavity. By these means different pressures have been recorded at different levels of the cavity. Thus around each catheter tip—irrespective of whether it is a small balloon, sponge tipped or an open end catheter—a small space is created filled by fluid. The pressure which is recorded from this space is the resultant of all contractile activities in the surrounding parts of the uterine walls.

The inertia or more accurately the frequency response of a fluid filled open end catheter system depends on among other things the length of the catheter, its diameter, the material of which it is made and the compliance of the pressure transducer. Recording of intra uterine pressures is complicated by the long narrow fluid filled catheters that must be used. Fig 3 indicates that the frequency response of the micro-transducer catheter is much better than that of the open end catheter. It is evident from Figs 4 and 5 that the frequency response of the open end catheter under optimal conditions is sufficient to record the intra uterine

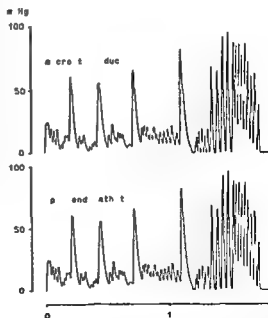


Fig 4 Comparison of pressure recordings *in vitro* by the micro-transducer catheter and the open end catheter system in the calibrator. The pressure fluctuations were brought about by compressing the inflated rubber balloon by hand. The recordings from the two catheter systems do not differ in any respect.

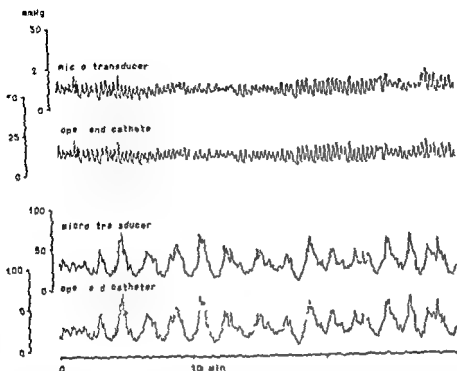


Fig 5 Comparison of simultaneous recordings of intra-uterine pressure by a micro-transducer and an open end catheter. The sensory surface of the microtransducer was kept from direct contact with the uterine walls by the arrangement shown in Text fig 1. The upper two tracings

represent recordings from a woman on day 14 in a normal cycle of 28 days. The lower two tracings are recordings from a woman on day 1 in a normal cycle of 25 days. In each pair of recordings the two pressure curves are practically identical.

pressure variations caused by the myometrial activity.

However, the frequency response of the open end catheter is easily decreased by clotting, air bubbles and leakage in the recording system, which can result in distorted recordings. When the micro-transducer is used, there is no risk of such disturbances of measurement. However, the results of the present study suggest that in order to give recordings in all respects similar to those obtained by open end catheters, the sensory surface of the micro-transducer catheter should be kept from a direct close contact with the uterine wall by some special arrangement. When the micro-transducer is used with an uncovered sensory surface permitting such a close contact with the uterine wall, the recordings show the same activity patterns as those obtained by other catheters, but the basal tone and the amplitude can deviate to a certain degree.

From our investigations it can thus be concluded that when correctly used, the micro-transducer catheter gives both reliable and reproducible recordings of the intra-uterine pressure. Such a re-

ording catheter has several advantages over previous methods. It is easily introduced through the uterine cervix. It is never obstructed. It has a frequency response far above that of fluid-filled open end catheters. It includes a standardised simple and reliable calibration of the whole recording system, including simultaneous disinfection of the recording catheter.

ACKNOWLEDGEMENT

We wish to thank Mr Lars-Göran Angenist, Department of Bio-engineering, Hospital of Lund, for his valuable technical advice.

REFERENCES

1. Åkerlund V, Bengtsson L, Ph & Carter A. A technique for monitoring endometrial or decidual blood flow with an intra-uterine thermistor probe. *Scand J Clin Lab Invest* 34: 469-477, 1975.
2. Asmussen M, Lundström A & Ulmsten U. Catheter manometer calibrator—a new clinical instrument. *Bio Med Eng* 175-180, 1975.
3. Asmussen M & Ulmsten U. Simultaneous

- urethro-cystometry and urethra pressure profile measurement with a new technique *Acta Obstet Gynecol Scand* 54 385-386 1975
- Bengtsson L Ph The sponge tipped catheter—a modification of the open end catheter for recording of myometrial activity *in vivo* *J Reprod Fertil* 11 115-118 1968
- Bengtsson L Ph & Negreiros de Paiva C (to be published)
- Brakema J T Janssens J Eskes T K A B & Hen II B Accurate pressure recording in the nonpregnant human uterus. A comparison of open and closed tip catheters *Europ J Obstet Gynecol* 6 195-206 1971
- Hendricks C H A new technique for the study of motility in the non pregnant human uterus *J Obstet Gynaecol Br Comm* 73 713-715 1964
- Jacobs R Killam H Barefoot C & Millar H Human application of a catheter with tip-mounted pressure and flow transducers *Rev Surg* 149-157 1977
- 9 Martinez Gaudio M Yoshida T & Bengtsson L Ph Propagated and nonpropagated myometrial contractions in normal menstrual cycles *Am J Obstet Gynecol* 115 107-111 1973
- 10 Nichols W W & Walker W E Experience with the Millar PC 350 catheter tip pressure transducer *Bio med Eng* 58-60 1974
- 11 Ulmsten U Studies on urethral function in women pp 1-115 Studentlitteratur Lund Sweden 1974

Submitted for publication Oct 26 1977

Mats Åkerlund
Department of Obstetrics and Gynecology
University Hospital
Lund
Sweden

CHANGES IN BONE MINERAL CONTENT IN WOMEN WITH NATURAL MENOPAUSE DURING TREATMENT WITH FEMALE SEX HORMONES

Nils Dalen, Marjam Furuhyelm, Bertil Jacobson and Bertil Lamke

From the Department of Medical Engineering, Karolinska Institutet, Department of Roentgenology, Karolinska Sjukhuset, and Department of Obstetrics and Gynecology, Sabbatsberg Sjukhus, Stockholm, Sweden

Abstract The bone mineral content was determined in eleven women with a natural menopause by X ray spectrophotometry during treatment with a combination of estrogens and a gestagen. During a three year follow up period the hormone treated women significantly increased ($P < 0.05$) their mineral content by 3% a year on average compared with a control group. Even in patients who had passed the menopause several years previously the increase occurred and was particularly great during the first year of treatment.

Postmenopausal women show a greater tendency to fractures than men of a comparable age. Albright et al. (7) observed this sex difference in fracture epidemiology and suggested that older women develop osteoporosis due to decreased ovarian function.

Although female sex hormones have been used for several decades, long term prospective studies on the effect of such treatment on the skeleton are few (1, 17).

The present study describes the changes in bone mineral content found in a group of women with natural menopause during treatment with female sex hormones.

MATERIAL AND METHODS

The material comprised women attending a gynaecologist for postmenopausal symptoms such as flushes, sweating, nervousness or for signs of atrophic vaginal and urethral mucosa. All the patients had passed the natural menopause at least two years previously and were in good clinical condition. The patients were treated with a combination of estrogens and gestagen according to the following schedule: Estradiol 4 mg + estrone 2 mg for 17 days followed by estradiol 4 mg + estrone 2 mg + norethisterone acetate 1 mg for 10 days followed by estradiol 1 mg + estrone 0.5 mg for 6 days. Blood estrogen was estimated at 6 months intervals. By this treatment blood

estrogen was kept at the same level as in the luteal phase of a normal menstrual cycle. The cyclic supplement with gestagen secured a regular shedding of the endometrium in all patients.

For ethical reasons the patients were not given placebo. Instead each patient was compared with a control of similar age. A selection was made to obtain controls with postmenopausal symptoms. In the original group of 14 patients, 3 were excluded as treatment was interrupted. The age of the hormone treated women was 56 ± 3 years and of the controls 55 ± 4 years (mean \pm S.D.).

The bone mineral content was determined by X ray spectrophotometry (7, 8). In this method different skeletal parts are positioned by TV fluoroscopy and automatically scanned by a beam from an X ray tube. The beam comprises two wavelengths by means of which it is possible to compensate for the attenuation in the soft tissues surrounding the bone. The mineral content was determined in the forearm (radius + ulna) 1 and 8 cm respectively proximal to the wrist.

Bone mineral per cent increase

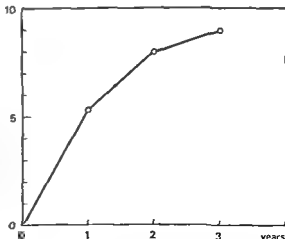


Fig 1 Difference in percentage change in bone mineral content between postmenopausal women treated with female sex hormones and controls. The plot represents the mean of 4 measuring sites.

Table I Bone mineral content in controls and in women treated with sex hormones at the start of the treatment

	Controls (mg/mm)		Hormone treated (mg/mm)		Difference		
	Mean	S D	Mean	S D	\bar{x}	t	P
Radius + ulna distal	101	22	107	24	+1	0.10	>0.05
Radius + ulna shaft	161	25	159	31	-1	0.17	>0.05
Femur neck	259	33	245	49	-5	0.79	>0.05
Femur shaft	474	57	438	60	-8	1.44	>0.05

mal to the radiocarpal joint) and the femur (neck and shaft). The sites of measurements and their precision have been described in detail by Dalén and Jacobson (5). Measurements were made at yearly intervals over a 3 year period.

The statistical evaluations were made according to Student's *t* test.

RESULTS

At the start of the study there was no significant ($P > 0.05$) difference in mineral content between women treated with sex hormone and controls (Table I). In the prospective part of the study the women treated with hormones increased their mineral content significantly ($P < 0.05$) by 3.3% on an

average per year as compared with the controls. There was no correlation between change of mineral content and age in either group. The greatest difference in change of mineral content between the two groups occurred at the beginning of the treatment (Fig. 1).

DISCUSSION

Albright et al. (2) proposed that deficiency of female sex hormones leads to osteoporosis by decreased bone formation. It has, however, later been shown

by analysis of the calcium metabolism (10) and by studies of microradiograms (12) that treatment with female sex hormones reduces the bone resorption. The current view is that estrogen inhibits the resorptive effect of parathyroid hormones (9).

By the development of new radiological methods, e.g. measurements of the bone mineral content, it has been possible to quantify skeletal changes. Alkvik et al. (1) have convincingly demonstrated that treatment with estrogen prevents the bone mineral losses after oophorectomy and even increases the bone mineral content when treatment is given within 3 years of oophorectomy. The bone mineral losses after oophorectomy are particularly high in trabecular bone that is in parts of the skeleton where older women show a high incidence of fractures (3, 6).

From a clinical point of view it is of particular interest to know whether treatment with female sex hormones can prevent the bone mineral losses normally occurring in women from their fourth decade. These physiological bone losses possibly have multifactorial etiology. For instance, older women often have a low calcium intake due to lactase deficiency (4). Therefore the conditions after artificial and natural menopause are not quite comparable.

In the present study the bone mineral con-

Table II Annual change in mineral content in controls and in women treated with sex hormones. The change has been expressed as a percentage of the initial values in the respective groups

	Controls (%)		Hormone treated (%)		Difference		
	Mean	S D	Mean	S D	\bar{x}	t	P
Radius + ulna distal	+1.7	7.6	+7.1	4.6	+5.4	7.03	>0.05
Radius + ulna shaft	-0.5	3.2	+7.0	4.6	+7.5	1.40	>0.05
Femur neck	+1.2	4.7	+4.7	5.1	+3.5	1.66	>0.05
Femur shaft	-2.9	2.5	-0.9	2.6	+1.9	1.75	>0.05
Mean of four sites	-0.1	3.9	+3.2	3.3	+3.3	2.16	<0.05

erred in those women treated with sex hormones. A similar result has been reported by Lehman et al (11) who found in 6 women with osteoporosis an increase of the bone mineral content during treatment with various estrogens during a 6-year period. In accordance with their findings the change in bone mineral content did not correlate with the age of the patients. Thus hormone treatment seems to have an effect on the skeleton even if the patient passed the menopause several years previously.

Riggs et al (12) have suggested that the effect of treatment with female sex hormones decreases with time as judged from findings on microradiograms. In long-term treatment the bone formation is suppressed after an initial inhibition of bone resorption. The shape of the curve in Fig. 1 is in agreement with the concept that the greatest effect is achieved at the beginning of the treatment.

REFERENCES

- 1 Aaker J M, Hart D M & Lindsay R. Oestrogen replacement therapy for prevention of osteoporosis after oophorectomy. *Br Med J* 3 515 1973.
- 2 Abtghil, F, Smith P H & Richardson A M. Postmenopausal osteoporosis: its clinical features. *J Am Med Assoc* 116 7465 1941.
- 3 Lehman P A & Bauer G C H. Epidemiology of fractures of the forearm. A biomechanical investigation of bone strength. *J Bone Jt Surg* 44 A 105 1962.

- 4 Birge S J Jr, Keutmann H T, Cantrecasas P & Whedon G D. Osteoporosis in estenal lactase deficiency and low dietary calcium intake. *New Engl J Med* 276 445 1967.
- 5 Dalen N & Jacobson B. Bone mineral assay—choice of measuring sites. *Invest Radiol* 9 174 1974.
- 6 Dalen N, Lamke B & Wallgren A. Bone-mineral losses in oophorectomized women. *J Bone Jt Surg* 56 A 135 1974.
- 7 Gustafsson L, Jacobson B & Kusoffsky L. X-ray spectrophotometry for bone mineral determinations. *Med Biol Eng* 12 115 1974.
- 8 Jacobson B. X-ray spectrophotometry in vivo. *Am J Roentgenol* 91 20 1964.
- 9 Heaney R P. Editorial. A unified concept of osteoporosis. *Am J Med* 39 877 1965.
- 10 Lafferty F W, Spencer G H Jr & Pearson O H. Effects of androgens, estrogens and high calcium intakes on bone formation and resorption in osteoporosis. *Am J Med* 36 514 1964.
- 11 Meema S, Bunker M L & Meema H E. Preventive effect of estrogen on postmenopausal bone loss. *Arch Int Med* 135 1436 1975.
- 12 Riggs B L, Sawsey J, Goldmith R S, Kelly P J, Hoffman D L & Arnaud C D. Short and long term effects of estrogen and synthetic anabolic hormone in postmenopausal osteoporosis. *J Clin Invest* 51 1659 1972.

Submitted for publication May 5 1977

Bertil Lamke
Department of Medical Engineering
Karolinska Institutet
S-10401 Stockholm
Sweden

ULTRASTRUCTURAL FEATURES IN NORMAL AND HYPERPLASTIC POSTMENOPAUSAL ENDOMETRIUM

Liane Deligdisch G Yedwab A Persitz and M P David

From the Governmental Municipal Hospital of Tel Aviv (Ichilov)
Tel Aviv Medical School Israel

Abstract Seven samples of postmenopausal endometrium were studied by electron microscopy. Four samples were diagnosed as adenomatous hyperplasia (2 of which were atypical) and 3 as normal postmenopausal endometrium. The most striking ultrastructural features of hyperplastic endometrium were numerous nucleoli, deep nuclear membrane infoldings, increased nucleocytoplasmic ratio, prominent and enlarged RER closely associated with mitochondria and nuclear membrane abundant free ribosomes and marked network microfilaments. In the case of atypical adenomatous hyperplasia the protruded intraglandular proliferating epithelial cells exhibited more atypical features than the epithelial cells of the glandular lining, suggesting a more advanced degree of atypical change. The characteristic features of the normal postmenopausal endometrium were paucity and random distribution of the cytoplasmic organelles, the presence of large cytoplasmic vacuoles and short blunt microvilli. Secretory vacuoles opening into the lumen of the gland were found in one case of cystic atrophy of a normal postmenopausal endometrium. Collagenization was found in the stroma of both groups, although predominantly in the normal postmenopausal endometrium. In 2 cases of adenomatous hyperplasia stromal cells with vacuolar cytoplasm were found. The significance of these changes as related to their importance as precursor stages of endometrial cancer (in the cases of adenomatous and atypical adenomatous hyperplasia) as involutional manifestations (in the cases of normal postmenopausal endometrium) and as related to the absence of cyclic activity (in both groups) is briefly discussed.

The precursor stages of endometrial carcinoma are now well documented (5). According to the classification of Velhio (10) adenomatous hyperplasia, atypical hyperplasia and carcinoma in situ represent the steps in the natural history of endometrial carcinogenesis.

The incidence of the precursor stages of endometrial carcinoma is high in postmenopausal women and few reports on the ultrastructure of the normal postmenopausal endometrium are published (1,2,3,4). A comparative study of the ultrastructural characteristics of normal and hyperplastic postmenopausal endometrium was undertaken.

MATERIALS AND METHODS

Samples of endometrium from 7 patients selected according to age group (47 to 52) and postmenopausal interval (2 to 4 years after onset) were studied by light and electron microscopy. The histopathological diagnoses were adenomatous hyperplasia in 4 cases, atypical adenomatous hyperplasia in 2 cases, inactive postmenopausal endometrium in 2 cases and inactive postmenopausal endometrium with cystic atrophy in one case.

The material was obtained by endometrial curettage followed by hysterectomy in the two cases of atypical hyperplasia of the endometrium. The histopathologic diagnosis was based on both endometrial curettings and samples obtained from the hysterectomy specimen.

The tissue was fixed in buffered glutaraldehyde for 2 hours, dehydrated in ethanol and embedded in Epon 812. Thick sections were first cut with the Porter Blum MT ultramicrotome and stained with toluidine blue in order to localize the areas chosen for ultrathin sections which were then stained with lead citrate and uranyl acetate. Observation sections and photography were carried out on JEM. The sections for light microscopy were fixed in formalin, sections of 4 μ m prepared in the usual way and stained with hematoxylin and eosin.

RESULTS

Light microscopy revealed the presence of crowded glands, intraglandular bridging and pluristratification of the tall columnar epithelium in the endometrial glands of all 4 cases diagnosed as adenomatous and atypical adenomatous hyperplasia. Cellular atypia was more marked in the 2 cases of atypical adenomatous hyperplasia in which numerous mitotic figures, nuclei with an irregular chromatin pattern and prominent nucleoli were found. Glands in glands, papillary projections and clusters of epithelial cells in the glandular lumen were frequently observed.

The normal postmenopausal endometrium was thin and the glands mostly small and lined by a single layer of cuboidal epithelium. Several dilated and cystic glands with an amorphous eosinophilic

Table I Nuclear ultrastructural changes in Endometria

	Nucleo-cytopl ratio	Nuclear infolding	Size of nucleoli	No of nucleoli	Perinuclear location of RER	Nuclear shape
107 AH	Moderately increased	Deep	Increased	3-4	Frequently present	Irregular or ovoid
101 PM	Decreased	Shallow	Normal or decreased	1-3	Absent	Round
104 PM	Decreased	No infoldings	Small to normal	1-2	Absent	Round
106 PM	Decreased	Shallow	Normal	1-2	Absent	Round-oval
107 AH	Increased	Deep	Increased	2-5	Present	Irregular or ovoid
109 AAH	Very increased	Deep	Increased	3-6	Present	Irregular
110 AAH	Increased	Very deep	Increased	4-5	Present	Irregular or ovoid

Table II Cytoplasmic ultrastructural changes in endometrium

AAH=atypical adenomatous hyperplasia AH=adenomatous hyperplasia PM=normal postmenopausal

	RER	Free ribosomes	Mitochondria	Lysosomes	Micro-filaments	Lateral membrane	Fat vacuoles
107 AH (48)	Very prominent dilated convoluted Related to nuclei and mitochondria	Very abundant	Numerous crowded Mainly sub nuclear	Numerous diffuse	Well developed	Slightly infolded	Absent
101 PM (50)	Poorly developed Unrelated to mitochondria	Relatively poor	Few random location	Rare	Almost absent	Indented or straight	Large light numerous pushing organelles
104 PM (50) cystic	Moderately developed Unrelated to mitochondria	Numerous	Numerous random location	Numerous apical area	Moderately developed	Indented	Numerous dilated and light secret into lumen
106 PM (48)	Well developed Unrelated to mitochondria	Poor	Moderately to numerous random location	Numerous mainly at apical border	Absent	Indented	Numerous secretion into lumen
107 AH (51)	Very prominent dilated related to mitochondria	Very numerous	Numerous random location	Poor	Well developed	Straight	Absent
109 AAH (47)	Very prominent & dilated related to mitochondria & nuclei	Very numerous	Numerous increased in size	Moderate	Well developed	Straight	Absent
110 AAH (49)	Very prominent & dilated In stacks parallel Related to nuclei and mitochondria	Generally abundant Reduced in some cells	Numerous sub and supra nuclear	Numerous	Well developed	Straight	Absent

Age of patient in parentheses

content were found in one case. The stroma was dense.

The ultrastructural changes of the nucleus and various cytoplasmic organelles are summarized in Tables I and II. The comparison between the major findings in the normal postmenopausal and the hyperplastic postmenopausal endometrium is summarized in Table III.

An obvious characteristic of the epithelial cells in the hyperplastic endometrium absent in the normal postmenopausal endometrium was the finding of prominent and convoluted Rough Endoplasmic Reticulum (RER) with a definite close relationship to mitochondria and nuclei (Fig. 1). Also found in the samples of hyperplastic endometrium was a delicate network of microfilaments found mainly perinuclear or between the mitochondria (Fig. 2). The nuclear characteristics found in all cases of

hyperplasia were deep indentations of the nuclear membrane, an increased number of nucleoli (2 to 6) and clumps of chromatin throughout the nucleus (Fig. 3).

The dominant characteristic of the normal postmenopausal endometrium was the presence of numerous vacuoles in the cytoplasm of the epithelial cells. These were clear, greyish or dark and pushed the cytoplasmic organelles to random locations (Fig. 5). Also found in the normal postmenopausal endometrium were epithelial cells with short blunt microvilli and an abundant collagen in the stroma. Collagen formation however was present also in 2 cases of hyperplasia (Fig. 6). Glycogen in the cytoplasm was poor or absent. A special feature was the frequent finding of secretory vacuoles opening into the glandular lumen (Fig. 7) in the sample in which light microscopy showed

	Glycogen	Stroma	Cilia	Microvilli	Protruding epithelial cells into glandular lumen
Normal	Relatively poor	Numerous collagen fibers Cells with fat droplets	Present	Normal length	-
Hyperplastic	Absent	Abundant collagen fibers	Present	Short blunt	-
	Absent	Abundant collagen	Absent	Normal length	-
Atrophic	Absent	Abundant collagen	Absent	Short blunt	-
Dysplastic	Present	Moderate collagen	Present	Normal length	-
Neoplastic	Present	Poor collagen abundant fat droplets	Present	Normal length	Present
	Relatively poor	Moderate collagen Numerous fat droplets	Absent	Normal length	Present

Table III *Comparative ultrastructural findings in normal and hyperplastic postmenopausal endometrium*
Nuclear characteristics

	Nucleo cytoplasmic ratio	Nuclear infolding	No of nucleoli	Perinuclear locations of RER	Nuclear shape
PM	Decreased (3)	Shallow (2) Absent (1)	1-2 (?) 1-3 (1)	Absent all	Round-oval all
AH & AAH	Increased (4)	Deep (4)	2-6 (4)	Present all	Irregular (1) oval (1)

Cytoplasmic characteristics

	PER	Free ribosomes	Mitochondria	Lysosomes	Microfil aments	Lateral Membranes	Fat vacuoles
PM (3)	Poorly developed (1) Moderately developed (2) Unrelated to mitochondria (3)	Numerous (1) Poor (?)	Numerous (1) Few (2) Random loca tion (3)	Numerous (2) Rare (1)	Absent (?) Moderately developed (1)	Infolded (3)	Present (1)
AH (1)	Increased Dilated	Very numerous (3)	Numerous (4) Mainly subnucl (3)	Numerous (?) Moderate (1)	Well developed (4)	Infolded (1) Straightened (3)	Absent (1)
AAH	Related to mitochondria (4)	Reduced (1)	Supranucl (1)	Rare (1)			

cystic atrophy of the endometrium. The nuclei were mostly round and contained 1-3 nucleoli.

A peculiar finding in the cases of atypical adenomatous hyperplasia was the protrusion into the glandular lumen of epithelial cells exhibiting an exaggeration of the characteristics found in the epithelial lining of the endometrial glands such as increased nucleo-cytoplasmic ratio, depth of nuclear infoldings, abundance of free ribosomes and RER. Also seen in these cells were straightened lateral cell membranes (Fig. 4).

DISCUSSION

Numerous detailed descriptions of the ultrastructure of the normal endometrial cycle (1, 2, 8, 11) of endometrial carcinoma (4, 9) and recently of endometrial hyperplasia (6) have been published. Descriptions of normal postmenopausal endometrium are few, however (7).

In our material the most prominent features of

hyperplastic endometrial cells were related to increased metabolic activity: increased protein synthesis and cellular replication, enlarged nuclei with deeply indented nuclear membrane, abundant convoluted and dilated RER with a peculiar close association to mitochondria and nuclear membrane. These features were absent in all cases of normal postmenopausal endometrium in which mitochondria were less abundant and randomly located, free ribosomes poorly represented and microfilaments poorly developed or absent. The nuclei in the normal postmenopausal endometrial cells were round with fewer nucleoli, suggesting that together with their cytoplasmic characteristics, a paucity of the organelles concerned with anabolic cell functions.

Degenerative changes also appear to take place in the cytoplasm of the epithelial cells of the postmenopausal endometrium. The finding of large vacuoles, probably fatty in nature, although the Sudan staining of the light microscopy sections was negative, occupying a large part of the cytoplasm and pushing the organelles (Golgi, mitochondria,

more advanced stage in the histogenesis of the endometrial cancer from its precursor stages

In one of the cases of atypical adenomatous hyperplasia in which such intraglandular protruding cells were found a hysterectomy was performed and an area of endometrial adenocarcinoma was found on random light microscopy examination (case 109)

The network of delicate microfilaments described as interconnecting the RER free ribosomes and perinuclear space is probably involved in cellular anabolic activity and active proliferation. It was absent in 2 of the 3 normal postmenopausal endometria and present in all of the 4 cases of adenomatous hyperplasia. These microfilaments were also found in endometrial carcinoma and described as unrelated to the degree of differentiation of the tumor (9).

Other ultrastructural features such as the presence of various lysosomes, the absence or poor representation of the Golgi apparatus and of the glycogen granules were found in both normal and hyperplastic postmenopausal endometrium and seems to be consistent with the lack of cyclic activity in both groups.

The presence of ciliated epithelial cells in some endometrial glands of both groups could be related to certain estrogenic influences (9) although no marked differences were found.

The stromal cells contained fat droplets in 2 of the hyperplastic postmenopausal endometrial samples which is consistent with the occasional finding of foamy histiocytes in the stroma of the atypical hyperplastic endometrium at light microscopy. This finding was considered as a reflection of hyperestrogenism.

There were no such stromal cells in the normal postmenopausal endometrium. Collagen formation in the stroma was marked in the normal postmenopausal endometrium and present in 2 hyperplastic postmenopausal samples and seems to be related to the absence of cyclic activity.

Although performed on a relatively small number of samples this study brought us an insight into some of the ultrastructural characteristics of a preneoplastic state (the adenomatous and atypical adenomatous hyperplasia) of the mechanism of evolution of an inactive tissue (the normal postmenopausal endometrium) and of ultrastructural features related to the absence of cyclic activity of the endometrium.

	Glycogen	Stroma	Cilia	Microvilli
1) Absent (3) urate		Abundant collagen (3) Lipids in cells absent (3)	Present (1) Absent (?)	Short blunt (?) Normal (1)
2) Present (?) der Rel poor (1) (?)		Abundant collagen (?) Poor collagen (?) Lipids in cells present (4)	Present (3) Absent (1)	Normal length (4)

etc.) to random locations could suggest the beginning of a cellular breakdown.

The finding of vacuolar structures opening into the glandular lumen in the case of cystic atrophy in a normal postmenopausal endometrium may illustrate the mechanism of formation of the cystic structures by an accumulation of amorphous material originating from cellular breakdown.

In both cases of atypical adenomatous hyperplasia areas of proliferating endometrial epithelial cells protruding into the glandular lumen were studied at the ultrastructural level. Two findings seem to be of interest.

1) A straightening of the lateral cellular membranes suggesting a morphological expression of the loss of contact inhibition by loosening of the intercellular connections which is characteristic of neoplastic growth.

2) An obvious exaggeration of the particular traits of the hyperplastic process in these cells suggesting that the protruded cells compared to those of the glandular lining are more anaplastic and represent a

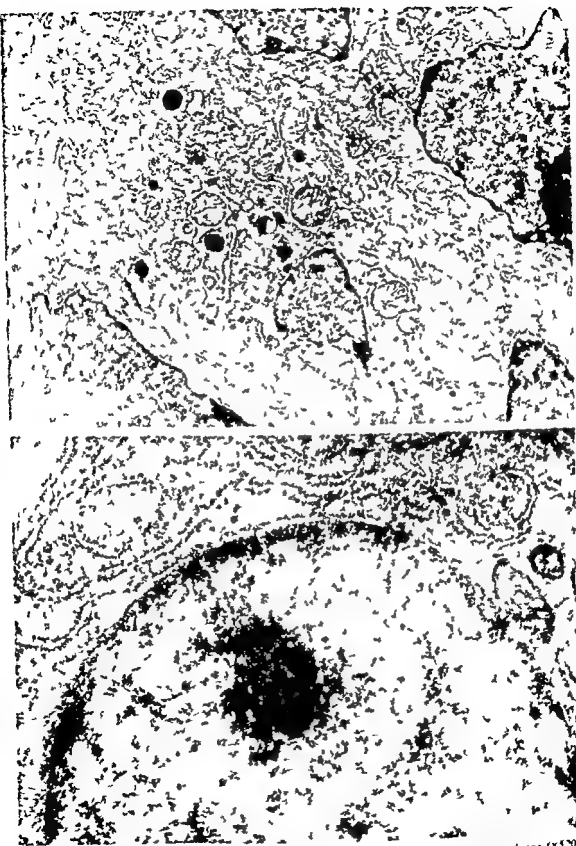


Fig 1 Top: Adenomatous hyperplasia. Prominent RER (rough endoplasmic reticulum) surrounding closely the mitochondria and the nuclear membrane ($\times 5000$). Bottom: Same cell higher magnification ($\times 8000$).



Fig. 2. Adenomatous hyperplasia. Microfilaments located between the nucleus and mitochondria ($\times 17\,000$).

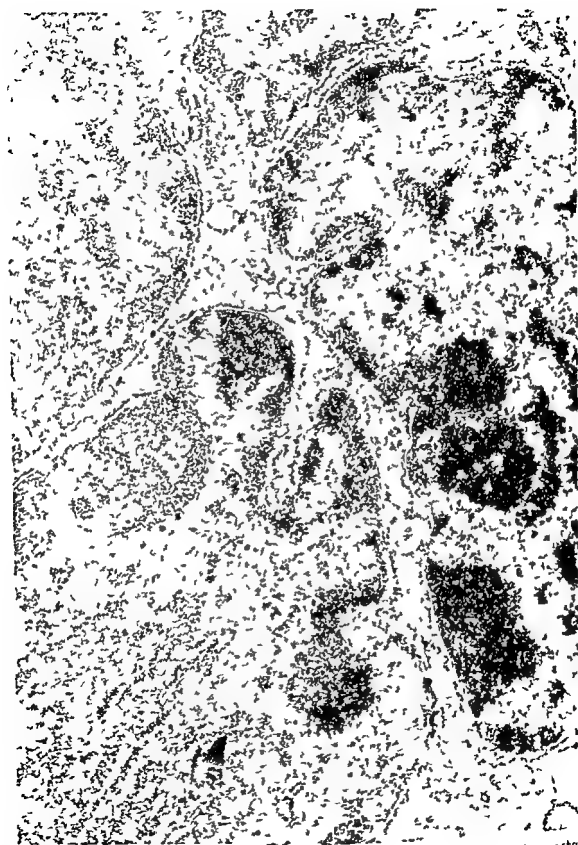


Fig. 3 Atypical adenomatous hyperplasia. Increased nucleocytoplasmic ratio, deep infoldings of nuclear membrane, irregular and coarse chromatin pattern ($\times 15\,500$).

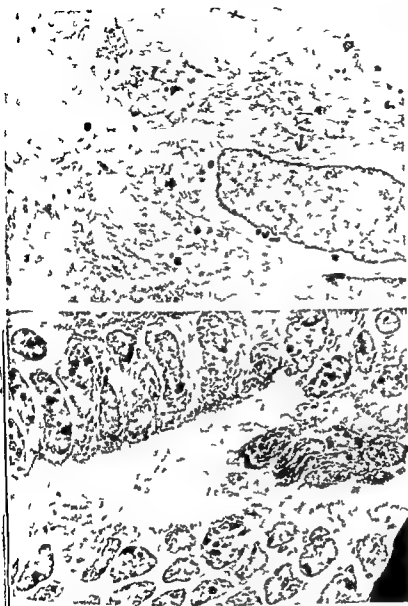


Fig. 4 Atypical adenomatous hyperplasia. Group of epithelial cells protruding into the endometrial lumen. Note the exaggeration of anaplastic features in this group of cells compared to the glandular lining, such as increased nuclear-cytoplasmic ratio, numerous nucleoli, coarse and irregular chromatin ($\times 3400$). Upper figure: an individual cell starting to protrude into the endometrial lumen. Arrow shows the straightened lateral cell membrane ($\times 7900$).



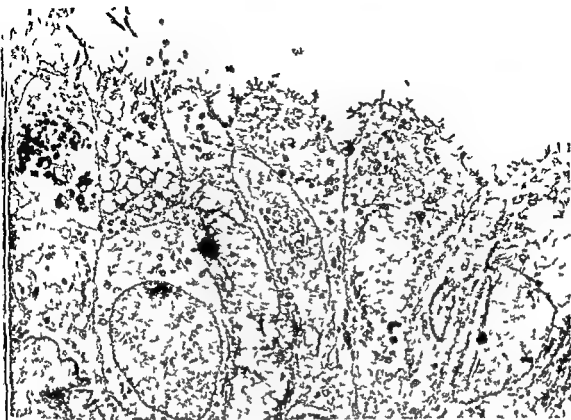


Fig. 5B Normal postmenopausal endometrium. The cytoplasmic vacuoles are partly dark and partly clear and push the cytoplasmic organelles to random locations. Note occasional ciliated cells. The microvilli are short and blunt ($\times 1,600$).

Fig. 5A Normal postmenopausal endometrium. The epithelial cells are cuboidal, the nuclei rounded with few nucleoli. The cytoplasm contains numerous clear vacuoles. In the stroma collagen is abundant surrounding stroma cells ($\times 15,300$).

REFERENCES

- 1 Cavazos F Ultrastructure of the human endometrial glandular cells during the menstrual cycle. *Am J Obstet Gynecol* 99: 833, 1967
- 2 Cavazos F Giant lysosomes and their associated structures in the normal human endometrium. *Am J Obstet Gynecol* 106: 1713, 1970
- 3 Ferenczy A & Richart R The Female Reproductive System. Dynamics of Staining and Transmission Electron Microscopy. Wiley, New York, 1974
- 4 Gompel Ch Ultrastructure of endometrial carcinoma. *Cancer* 38: 745, 1973
- 5 Gusberg S B Ultrastructural and neoplastic endometrium. *Am J Obstet Gynecol* 116: 175, 1973
- 6 Richart R & Ferenczy A Endometrial morphologic changes in the hormonal environment. *Gynecol Oncol* 2: 1, 1973
- 7 Saxena S C Ultrastructure of endometrium in postmenopausal women. *Annals of the 16th All India Obstet Gynaecol Congress* 1972 pp 105-106
- 8 Schneller Ed Ultrastructure of cultured cells in human endometrium. *Obstet Gynecol* 41: 188, 1973
- 9 Trasler T & Richart R An ultrastructural comparison of endometrial carcinoma and normal endometrium. *Cancer* 29: 1713, 1973
- 10 Velhio F Endometrial hyperplasia and carcinoma in situ. *Gynecol Oncol* 2: 15, 1974
- 11 Wienke et al Ultrastructure of the human endometrial stromal cell during the menstrual cycle. *Am J Obstet Gynecol* 107: 65, 1968

Submitted for publication April 1, 1977

Liane Dehgdisch
The Mount Sinai Hospital
Fifth Avenue and 100th Street
New York
NY 10029
USA

VULVOVAGINAL CANDIDIASIS IN PREGNANCY TREATED WITH CLOTRIMAZOLE

Kjell Haram and Ashbjørn Digranes

From the Department of Obstetrics and Gynecology and the Gade Institute Department of Microbiology University of Bergen Bergen Norway

Abstract An open trial of local clotrimazole therapy in 56 pregnant women with vulvovaginal candidiasis is reported. The diagnosis was confirmed by mycotic culture. The patients were given one vaginal tablet daily and cream was applied to the vulva 2 or 3 times daily. Their male partners were treated with cream only. The duration of therapy was 6 days. Fifty of the patients (89.3%) were clinically cured after 6 days of therapy. Six patients (10.7%) had slight complaints and 10 (17.9%) without symptoms or signs of infection harboured *Candida albicans* or other yeast species in the genital tract. Six of the patients were given a second treatment with clotrimazole and their remaining symptoms subsided. Candidiasis recurred later in pregnancy in 4 of the 56 patients studied. The implications of the presence of *Candida* in the genital tract are discussed. It is concluded that clotrimazole is an effective antimycotic agent which can be used for vulvovaginal candidiasis during pregnancy without causing side effects. Two of the patients had trichomoniasis concurrently. One of these was cured with clotrimazole.

MATERIAL AND METHODS

Fifty six pregnant women were investigated. Age, length of gestation and duration of symptoms at the start of therapy are shown in Table I. Seven patients had had some other form of antimycotic treatment earlier during the pregnancy. None of the patients were diabetics. All had vaginal discharge, itching and burning pain in the vulva and obvious vulvar inflammation. High vaginal swabs were inserted into Stuart's transport medium and brought to the microbiological laboratory. The specimens were cultured on Sabouraud dextrose agar. The plates were incubated at 25°C for 4 days. Isolates of *Candida albicans* were identified by the production of germ tubes in human serum (5). Other yeast species were not identified. All specimens were also examined for *Trichomonas vaginalis* using Diamond's substrate (3) as the culture medium. After inoculation the tubes were incubated at 37°C. Microscopical examination of material from the tubes was performed after 2 days and when found negative after a further 3 days.

The patients were treated with one clotrimazole (100 mg) vaginal tablet daily for 6 days and in addition cream was applied to the vulvar area 2 or 3 times daily. Their male partners were not examined but were treated with clotrimazole cream. The patients were advised not to have sexual intercourse during this period.

The women were examined 3 weeks after the commencement of treatment. Patients with persistent vulvitis were given an additional period of treatment with clotrimazole. Those without symptoms were checked with a second mycotic culture.

The patients were followed until parturition but no further mycotic culture was done and the patients were not examined in the puerperium. Recurring vulvovaginal candidiasis was however treated with the same agent.

Table I Clotrimazole therapy in 56 pregnant women with vulvovaginal candidiasis. Age, length of gestation and duration of complaints

	Mean (range)
Age (years)	27.1 (17-36)
Gestation length (weeks)	4.7 (8-33)
Duration of complaints (weeks)	11.4 (1-33)

An association has been reported between pregnancy and impaired immunological function (6, 8, 10, 11, 15). Cellular immunity in the host is fundamental for the defence against viral and fungal diseases. Vulvovaginal candidiasis is common during pregnancy and it is conceivable that cellular immunity is involved. In addition, it is thought that an action of oestrogen from the placenta on carbohydrate metabolism promotes the growth of yeast in the lower genital tract (1, 2).

The presence of *Candida* does not indicate manifest candidiasis (11). However, inflammatory changes and particularly obvious vulvitis represent definite fungal disease.

As pregnancy predisposes to candidiasis and treatment is indicated, it is valuable to test the effect of antimycotic agents in pregnant women.

The purpose of the present study was to investigate the effect of clotrimazole on manifest vulvovaginal candidiasis in pregnancy using the recommended medication.

Table 1 Bladder pressure (BP) maximum urethral pressure (MUP) closure pressure (CP) and functional length of the urethra (FUL) in 16 healthy females at a bladder volume of 200 ml

Patient	BP (cmH ₂ O)	MUP (cmH ₂ O)	CP (cmH ₂ O)	FUL (mm)
1	6	46	40	24
2	20	96	76	38
3	20	108	88	30
4	20	95	75	30
5	28	136	108	30
6	16	75	59	25
7	24	96	72	28
8	24	115	91	32
9	18	97	79	32
10	20	106	86	41
11	20	57	37	37
12	10	101	91	28
13	20	60	40	34
14	20	91	71	33
15	8	77	49	26
16	28	77	49	26
Mean	20	90	69	31
Range	6-28	46-136	37-108	24-42

transducer of the recording catheter was placed at that site in the urethra of maximal intra urethral pressure (Fig. 1). The infusion of saline was then continued at the same rate.

During the simultaneous recording of the intravesical and urethral pressures at increasing bladder volume the women were requested to announce when they had sensations of urgency and wanted to void. At that

time the infusion catheter was withdrawn and the women were allowed to micturate.

The recordings were performed with the subjects in the sitting position.

For the first five examinations the abdominal pressure was recorded by means of an intrarectal micro-transducer. Simultaneous bladder and urethral pressures were registered to obtain a true detrusor pressure. Since the intrarectal pressure recording apparently did not give additional information it was not used in subsequent investigations.

After the investigations were completed the residual urine was measured and a sample sent for bacterial culture. These tests were repeated on the 10th day after the investigations.

Before and after the examination the recording equipment including the catheter was calibrated as described previously (Asmussen, Lindstrom & Ulmsten 1976).

Recorded parameters

The following parameters were measured (see Tables I-IV and Figs 1-4): bladder pressure, maximum urethral pressure, urethral closure pressure, i.e. the difference between maximum urethral pressure and the bladder pressure, bladder volume at voiding, detrusor latency time, i.e. the time interval between the decrease in urethral pressure and detrusor contraction (Fig. 2), pre voiding time, i.e. the time interval between the decrease in urethral pressure and zero closure pressure (Fig. 3), the residual urine after voiding.

RESULTS

The findings in the healthy females are given in Tables I and II and in Fig. 2. As can be seen when

Table II Bladder pressure (BP) maximum intra urethral pressure (MUP) and closure pressure (CP) just before initiation of voiding, bladder volume (BV) at voiding, detrusor latency time (dlt), pre voiding time (pvt) and residual urine (RES) after voiding in 16 healthy females

Patient	BP (cmH ₂ O)	MUP (cmH ₂ O)	CP (cmH ₂ O)	BV (ml)	dlt (sec)	pvt (sec)	RES (ml)
1	0	76	56	400	0.8	4.8	0
2	24	128	104	500	3.0	10	0
3	24	104	80	475	1.4	2.0	1
4	32	104	72	450	4.6	6.0	2
5	28	136	108	375	1.2	4.8	6
6	16	79	59	375	2.0	4.0	2
7	8	96	68	250	2.5	2.5	2
8	32	148	116	350	5.0	1.2	1
9	36	140	104	500	2.5	7.0	0
10	4	114	90	350	4.0	6.0	1
11	4	69	45	350	4.0	8.0	1
12	0	112	92	400	2.5	3.0	0
13	24	72	48	400	1.6	11	6
14	24	91	67	500	6.4	8.4	3
15	40	97	57	300	2.0	4.0	5
16	60	117	57	450	2.4	3.6	4
Mean	9	105	76	402	3.0	6.2	1
Range	0-60	69-149	45-116	250-500	0.8-6.4	2.0-12	0-6

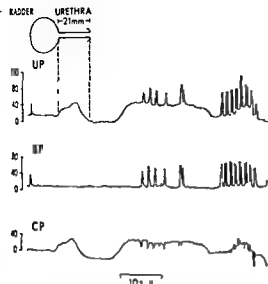


Fig 1 Simultaneous recordings of urethral pressure (UP) bladder pressure (BP) and closure pressure (CP) i.e. the difference between urethral pressure and bladder pressure. Left part of the tracings recording of the urethral closure pressure profile. Right part of the tracings recording of pressure changes during repeated coughing

the bladder filling increased from 200 ml to the volume III which voiding occurred there was a mean increase in bladder pressure from 20 cm H₂O to 29 cmH₂O and in maximum intra urethral pres



Fig 2 Recordings of urethral pressure (UP) bladder pressure (BP) and closure pressure (CP) in a healthy woman at the initiation of voiding. dlt=detrusor latency time pvt=pre voiding time

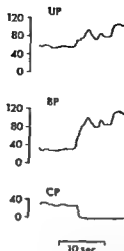


Fig 3 Recordings of urethral pressure (UP) bladder pressure (BP) and closure pressure (CP) in a woman initiating voiding by the Valsalva manoeuvre

sure from 90 cmH₂O to 105 cmH₂O. Immediately before voiding the maximum intra urethral pressure decreased. A few seconds later (mean 3.0 sec see dlt in Table II) the intravesical pressure increased. As a result of these pressure changes the urethral closure pressure decreased to zero. This occurred after a mean of 6.2 sec (see pvt in Table II) and at this time urine started to escape the urethra (Fig 2). The bladder volume at which the women wanted to micturate varied between 250 ml and 500 ml (mean 400 ml) and they were all able to empty their bladders completely (Table II).

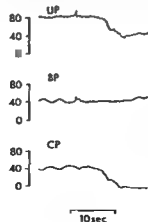


Fig 4 Recordings of urethral pressure (UP) bladder pressure (BP) and closure pressure (CP) in a woman initiating voiding mainly by decreasing the urethral pressure

Table III Bladder pressure (BP) maximum urethral pressure (MUP) closure pressure (CP) and functional length of the urethra (FUL) in 13 patients with stress incontinence at a bladder volume of 200 ml

Patient	BP (cmH ₂ O)	MUP (cmH ₂ O)	CP (cmH ₂ O)	FUL (mm)
1	27	63	36	22
2	16	44	28	32
3	29	55	55	33
4	6	66	60	34
5	8	48	40	30
6	20	44	24	16
7	24	61	37	44
8	3	72	40	29
9	28	84	56	30
10	32	92	60	28
11	24	50	26	20
12	1	60	48	13
13	12	44	32	31
Mean	21	63	42	29
Range	6-32	44-92	24-60	16-44

The results obtained in the stress incontinent patients are shown in Tables III and IV and in Figs 3 and 4. The mean maximum urethral pressure and the mean closure pressure at a bladder volume of 200 ml were lower in these patients than in the healthy women. However the mean bladder pressure was the same 20 cmH₂O. When the bladder volume increased from 200 ml to the volume at

which voiding occurred the intravesical pressure increased to 29 cmH₂O. In contrast to what was found in the normal women there was a simultaneous increase in maximum urethral pressure. In 5 of the patients (Nos 1-5 in Table IV) micturition was initiated by the Valsalva manoeuvre (Fig 3). Three patients (Nos 6-8 in Table IV) initiated voiding mainly by decreasing the maximum urethral pressure (Fig 4). The remaining patients (Nos 9-13 in Table IV) initiated voiding mainly in the same way as the healthy females: a bladder volume at which the patients wanted void varied between 300 ml and 800 ml (mean 400 ml) and some of them had difficulty in emptying their bladders completely (Table IV).

DISCUSSION

It must be emphasized that voiding in a laboratory implies an unpleasant situation for a female. Great efforts should therefore be made to allow the patient to adapt to the recording situation and the measuring equipment used should be as easily handled as possible in order not to influence the responses. The recording technique used in this study allowed 29 out of 32 patients to void promptly and as they said in their normal way during the investigations.

Most studies of micturition in the female

Table IV Bladder pressure (BP) maximum intra urethral pressure (MUP) and closure pressure (CP) before initiation of voiding bladder volume (BV) at voiding detrusor latency time (dlt) pre voiding (pvt) and residual urine (RES) after voiding in 13 women with stress incontinence

Patient	BP (cmH ₂ O)	MUP (cmH ₂ O)	CP (cmH ₂ O)	BV (ml)	dlt (sec)	pvt (sec)	RES (ml)
1	35	63	8	400	-	0.8	45
2	36	44	8	500	-	0.8	30
3	36	55	52	700	-	1.2	40
4	18	66	48	500	-	0.2	1.5
5	10	48	38	300	-	0.8	80
6	24	44	20	325	2.0	2.0	3
7	28	49	21	300	0.8	0.8	1
8	36	48	48	325	4.0	3.2	4
9	3	72	40	300	9.6	14	10
10	5	80	28	400	1.0	1.6	8
11	28	58	30	300	0.8	4.8	1
12	28	40	12	800	1.0	3.0	60
13	20	44	24	350	1.2	4.4	20
Mean	29	60	31	423	2.6	3.7	31
Range	10-32	40-88	8-52	300-800	0.8-9.6	0.2-14	

carried out as pressure flow studies which means recording the intravesical pressure and the flow of urine through the urethra (see Hjälmås 1976). Doubtless such a recording technique gives valuable information about micturition in the male, but it seems to be of minor value in the female in whom outflow obstructions are less frequent. Furthermore, it does not permit detailed studies of the interplay between the urethra and the bladder at the very initiation of micturition. In the few studies of micturition in females performed with simultaneous intra urethral and intravesical pressure recordings, relatively wide bore catheters have been used (Karlsson 1957; Enhörning 1961). A urethral catheter always implies a risk of disturbing normal micturition; in particular, there is a risk of obstructing the flow of urine through the urethra, as has been shown that the urethral lumen during micturition seldom exceeds 3–4 mm (Smith 1968). If the recording catheter used in this study has an inner diameter of 1.6–2.2 mm, it should reasonably be a minimal obstruction to the flow of urine.

Technically, the present recording equipment has important advantages compared with previously used techniques for study of the initiation of micturition in females. It is easy to calibrate and to handle. It measures the intravesical and the intra urethral pressure as well as the urethral closure pressure related to atmospheric pressure, and no fluid is necessary for the recordings.

Compared with balloon catheters and open end catheters, the micro-transducer catheter has a better frequency response. The micro-transducer can adequately record pressure changes occurring faster than 10 msec (Asmussen, Lindström & Ulmsten 1975), which is more than sufficient for the changes occurring in the lower urinary tract.

The present investigation confirms a preliminary report concerning initiation of voiding in healthy females (Ulmsten, Andersson & Persson 1975). Thus, in the healthy females, micturition commenced by an initial decrease in the urethral pressure, followed by increase in the intravesical pressure. Generally speaking, the urethral closure pressure reached zero within 6–7 seconds after the females were allowed to void.

The cause of the observed pressure changes can not be determined by the present investigation, but there is reason to believe that the rapid initial decrease in urethral pressure was mainly caused by a voluntary relaxation of the striated muscles in the

pelvic floor, partly supplying the urethra (Öbrink, Ingelman Sundberg & Ulmsten 1977a), whereas the increase in bladder pressure indicated activation of the detrusor.

Influences from the autonomous nervous system might also contribute to the actual pressure changes. Thus, in cats, an increased activity in the sympathetic nervous system has been shown during the filling phase of the bladder (Edvardsen 1968). Stimulation of beta adrenoceptors predominating in the bladder wall diminishes the increase in bladder pressure obtained when increasing bladder volume (Edvardsen 1968). Conversely, stimulation of the alpha adrenoceptors, which predominate in the urethra (Ek, Alm, Andersson & Persson 1977), increases the maximal intra urethral pressure (Ek, Andersson & Ulmsten 1977). The intra urethral pressure in healthy females also increased with increasing bladder volumes, which finding tallies with previous findings (Ulmsten et al. 1975; Öbrink, Ingelman Sundberg & Ulmsten 1977b).

Thus, an interruption of the sympathetic nerve stimulation to the urethra and bladder at initiation of micturition may contribute to a decrease in the intra urethral pressure and facilitate an increase in the intravesical pressure. In the present investigation, eight stress incontinent patients initiated micturition by decreasing the urethral pressure only or by a Valsalva manoeuvre. The reasons for these modes of initiating voiding are unclear.

Despite the small number of women investigated in this study, the results make it of interest to discuss the consequences of surgical treatment of stress incontinent patients. Thus, in patients who initiate voiding by decreasing the urethral pressure, only operative procedures with non absorbable slings should be carried out with caution, since relaxation of the urethra might be impaired with a consequent increased risk of postoperative incomplete voiding. Similarly, overcorrection with traditional urethropexies using unabsorbable sutures may increase the risk of postoperative retention of urine. In patients voiding by means of a Valsalva manoeuvre, a sling could also complicate micturition, since straining of the abdominal muscles to which the slings are attached may also cause a strain in the sling, leading to a further elevation of the urethra at initiation of micturition. However, to estimate with certainty both qualitative and quantitative disturbances of voiding after different surgical procedures in women suffering from

continence analyses of micturition must be done before as well as after the actual surgical procedures.

REFERENCES

1. Asmussen M, Lindström K & Ulmsten U. A catheter-manometer calibrator—a new clinical instrument. *Biomed Engin* 10: 175 1975.
2. Asmussen M & Ulmsten U. Simultaneous urethrocytometry with a new technique. *Scand J Urol Nephrol* 10: 7 1976a.
3. Asmussen M & Ulmsten U. A new technique for measurements of the urethral pressure profile. *Acta Obstet Gynecol Scand* 55: 167 1976b.
4. Alm P, Andersson K E & Persson C G A. Adrenoceptor and cholinergic mediated effects in the isolated human urethra. *Scand J Urol Nephrol*. In press.
5. Andersson K E & Ulmsten U. The effect of norephedrine and bethanechol on the human urethral pressure profile. *Scand J Urol Nephrol*. Submitted for publication.
6. Fitzmaurice G E. Simultaneous recording of intravesical and intraurethral pressure. A study on urethral closure in normal and stress incontinent women. *Acta Clin Scand* Suppl 176: 1 1961.
7. Edvardsson B. Nervous control of urinary bladder in cats. I. The collecting phase. *Acta Physiol Scand* 72: 157 1968.
8. Hjalmas K. Micturition in infants and children with normal lower urinary tract. A urodynamic study. *Scand J Urol Nephrol* Suppl 37: 1976.

9. Karlsson S. Experimental studies of the function of the female urinary bladder and urethra. *Acta Obstet Gynecol Scand* 32: 285 1953.
10. Smith J C. Urethral resistance to micturition. *Scand J Urol* 40: 125 1968.
11. Ulmsten U, Asmussen M & Lindström K. A new technique for simultaneous urethrocytometry and measuring measurements of the urethral pressure. 14th Annual Meeting of the International Continence Society, Glasgow 1975. *Urologia Int* 32: 177 1977.
12. Ulmsten U, Andersson K E & Persson C G A. Diagnostic and therapeutic aspects of urinary incontinence in women. 14th Annual Meeting of the International Continence Society, Glasgow 1975. *Urologia Int* 32: 88 1977.
13. Öbrink A, Ingelman Sundberg A & Ulmsten U. The urethral pressure profile before, during and after pubococcygeal repair for stress incontinence. *Acta Obstet Gynecol Scand* 57: 49 1978a.
14. Öbrink A, Ingelman Sundberg A & Ulmsten U. A study on the urethral pressure profile in continent women. In press.

Submitted for publication June 20 1977

Ulf Ulmsten
Department of Obstetrics and Gynecology
University Hospital of Malmö
S 21401 Malmö
Sweden

BLADDER BASE INSUFFICIENCY

Radiological Urodynamic and Clinical Aspects

Knud P. Olesen and Steen Walter

*From the Department of Radiology and the Department of Urology
Gentofte Hospital University of Copenhagen DK 2900
Hellerup Denmark*

METHODS AND MATERIALS

Colpo cysto-urethrography (a voiding cysto-urethrogram with additional contrast medium in the vagina (Olesen & Walter (19)) was performed on 470 patients from the urological laboratory including all patients referred to the department of gynecology for utero-vaginal prolapse conditions with or without urinary symptoms. A urodynamic and gynecological examination including a careful interview was performed on a separate occasion and not disclosed until a radiological diagnosis had been reached.

FINDINGS

The alteration in bladder base morphology that was termed bladder base insufficiency (b b i) in a previous report by Olesen (17) was found in 110 patients (26.2%). The base is pointed with the internal urethral orifice situated at the lowest point of the bladder and lower and more anterior than normally. This was diagnosed if the orifice was below the lower margin of the symphysis and the distance from the symphysis to the orifice less than 20 mm. The outline of the vagina was normal with an unaltered axis and no bulgings (Fig. 1b). The description refers to the picture of the resting bladder filled to the patient's normal desire to void. This view is all that is needed for the diagnosis.

In some cases the picture during cough revealed incontinence; in other cases only an increase in the pointedness of the bladder base. Even though a patient may suffer from stress incontinence this is not necessarily shown radiologically nor registered by the flowmeter because the cough is not spontaneous. The patient is en garde. We have seen this in a patient who did not show incontinence during the premeditated cough but immediately afterwards had a spontaneous cough resulting in incontinence.

The radiological signs of a normal

Among 40 consecutive patients referred for voiding cystourethrography (16) presented the picture of bladder base insufficiency (b b i). The examination was done as a colpo-cysto-urethrography. A urodynamic and gynecological examination was performed in each patient. The characteristic morphological features were: anterior and inferior displacement of the bladder neck and pointed bladder base. The position and form of the vagina was normal. Radiological signs of detrusor function were weak and opening of the bladder neck was characterized by funneling. Urodynamically the patients with b b i showed low opening and low detrusor contraction pressures. The flows were highly varying. Very high flows were seen in a few patients but the more common pattern was slightly reduced maximum flow rates. Opening of the internal urethral orifice is known to be caused by detrusor contraction. Closing is passive, caused by elastic properties in the tissues. In b b i, intravesical pressures during micturition were low and radiological signs of detrusor contraction were weak, indicating that the bladder neck was easily opened. This on the other hand means that the bladder neck was insufficiently closed during bladder reservoir function and may explain the main symptom: stress incontinence, which was present in 84% of the patients. The underlying pathology in anatomical support and suspension of the bladder base is discussed.

In the literature on female stress urinary incontinence certain clearly described morphological changes of urethra and bladder pervade. Mueliner (16), Ball et al. (3), Roberts (21) and Ala-Ketola (1) among many others have described low position and funneling of the bladder base. Increase of the posterior urethro-vesical angle was reported by Jeffcoate & Roberts (13), Green (7) and Palm (20). Discussed alterations in urethral inclination. Hutch (17) stated that the base of the continent bladder is flat. Many authors have confirmed these observations although a few have challenged one or another of the findings. A few authors have doubted the value of these criteria when applied to their own examinations. Hoffmann & Ulrich (11), Zachann (14).



Fig. 1. Resting, filled bladder: (a) Normal case; (b) Bladder neck insufficiency.

micturition are rounding of the bladder, serration of the bladder wall above the ingone and a well defined shelf-like horizontal part of the bladder base in front of the internal urethral orifice as shown in Fig. 2a. It is customary to describe the opening of the bladder neck during normal micturition as a funneling. This term, however, is not appropriate and may lead to misunderstanding. In normal cases there is no funneling of the base until the last part of the micturition.

In bladder base insufficiency, however, opening of the bladder neck involves funneling as shown in Fig. 2b. The absence of the anterior flat part is typical. Rounding of the bladder is the only constantly present sign of detrusor function. Serration of the bladder wall is rare and slight. The urethra is usually slender, tapering evenly to the external striated sphincter. Variations in calibre are seen from one micturition to another and during the progress of the same micturition. The urethro-pelvic angles described by Olesen & Walter (19) are normal.

The above described micturition was seen in 68 cases. Among the remaining patients 22 showed a sliding of the entire bladder base down in front of the vagina, an anterior or prevaginal bladder descensus. The urethro-pelvic angles were subnormal, that is below 70°. This produced an eversion of the anterior vaginal wall into the introitus, but no

alteration in vaginal axis or position. This anterior bladder descensus was most pronounced in 16 and sometimes disappeared completely towards the termination of micturition. Most often the intravaginal pressure measurement showed this condition to be associated with straining. Another 16 patients showed a posterior descensus position during micturition, that is with a displacement of the vagina in a postero-inferior direction and a central vaginal bulge. Two patients could not micturate during the examination and 2 showed signs of previous operations.

URODYNAMICS

Cystometrically 4 patients displayed uncoordinated detrusor contractions indicating a suprasacral neurogenic bladder paresis according to Hald (14). Their symptoms were dominated by urge incontinence. The remaining patients had normal cystometrograms.

To describe the urodynamic picture in pure bladder base insufficiency we excluded all patients who demonstrated a descensus during micturition in the radiological examination. The 4 patients with a coexistent neurogenic dysfunction are also excluded. Two of the remaining 64 patients did not go through a full urodynamic examination.

The urodynamic values are calculated as the



Fig. 1 Micturition (a) Normal case (b) Bladder base insufficiency

the values of 3 micturations the variations in each test are not shown.

The pressure rise at the start of the micturition is described as opening pressure minus pre-micturition pressure. The values are shown in Fig. 2. The average is slightly lower than normal values published by Zinner & Paquin (25), Backman et al. (7), Palm (20) and Frimodt Møller (6). The detrusor contraction pressure is defined as mean micturition pressure minus pre-micturition pressure. The values are shown in Fig. 4. Zinner & Paquin and Frimodt Møller (6) gave normal values. In the pressure values in b b 1 are lower than normal. Uro-flow is determined as the maximum rate and displayed in Fig. 5. These values were in the normal range.

Bladder volumes varied from 175 to 500 ml. The duration of micturition (flow time) was extremely variable. The lowest was 12 sec at a bladder volume of 170 ml. The highest was 66 sec at a volume of 450 ml.

CLINICAL FINDINGS

The gynecological examination revealed some laxity or bulging of the anterior vaginal wall in 70 patients. Vaginal repair operations had been performed in 25 patients. One patient had had an hysterectomy. Hysterectomies had been performed in 15 cases. Age and parity distribution (Fig. 6) was

an almost exact replica of the distribution of all 420 patients.

Stress incontinence was a symptom in 92 patients (83.6%). Urge incontinence was the major complaint in 11 patients. Two patients gave straining as their main symptom while one suffered from recurrent cystitis. Nine of the patients had no urological symptoms.

DISCUSSION

In the literature the pointed appearance of the bladder base is widely described as funneling. The low and anterior position of the bladder neck also con-

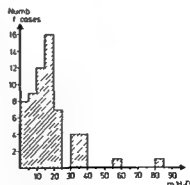


Fig. 3 Bladder base insufficiency. Opening pressure minus pre-micturition pressure \bar{x} $17.0 \pm S.D. 13.7$ cmH₂O. 67 cases.

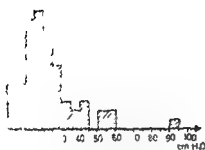


Fig. 5 Bladder base insufficiency. Detrusor contraction (maximum intravesical pressure minus premitigation) 19.3 ± 8.9 cmH₂O. 63 cases

in with the findings of numerous previous authors (23) Hodgkinson (9-10) and Alajaloja (11) surveys of this literature. We found a normal position and course of the urethra in accordance with Green's type I or a retraction of the bladder during micturition which terms with Green's type II (7). We did not measure the posterior urethro-vesical angle because we have found it to vary from 100° to 180° in continent women. In b b₁ however the downward forward sliding of the bladder base in front of the unaltered vagina necessarily tends to increase the angle which is in accordance with the original observation made by Jeffcoate & Roberts (13).

Furthermore we agree with Béthoux et al (4) who on the basis of colpocystograms describe 2 bladder suspension defects: type A that leaves the

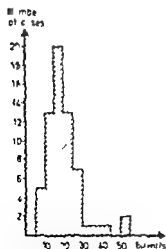


Fig. 6 Bladder base insufficiency. Age and parity distribution. 110 cases

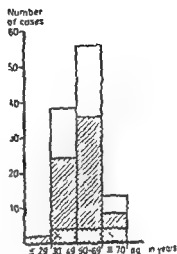


Fig. 7 Bladder base insufficiency. Age and parity distribution. 110 cases

vagina unaffected and type P that produces a large bulge of the anterior vaginal wall but no retraction in vaginal position. Lazarevski et al (14) confirm these findings and conclude that the 2 types A and P correspond to Green's types I and II.

The radiological signs suggest weakness of the function in b b₁. This is in accordance with the urodynamic finding of rather a low opening pressure and detrusor contraction pressure and indicates that little effort is needed for the active opening of the bladder neck. On the other hand it may also account for the major symptom of these women: Stress incontinence. The bladder neck has reduced resistance to passive opening during increases in abdominal pressure.

Why is the closing of the internal urethral orifice insufficient? Opening of the orifice is active and is caused by detrusor contraction. Closing is passive and is caused by a distinct circular sphincter exists as shown in cross sections by Kranitz (14) and Tanagho & Smith (15). Such a passive closing mechanism cannot be expected to possess any high resistance to opening unless properly supported from the outside.

Béthoux & Bory (4) make comparisons with quadrupeds. In these the major function of the levator muscle is to wag the tail. It has no other function as support for the pelvic organs. The suspension in the quadrupeds of the bladder is effected by the fascial layers of the pelvis and the

traction exerted on the bladder neck is dorsal in direction. In the human the levator muscles have assumed a very definite function as support for the pelvic organs. By forming a sling behind the vagina they supply the postero-inferior support of vagina and bladder. On contraction the levator lifts the vagina and bladder in antero-cranial direction. The anatomy of the levator however does not prevent the bladder base from sliding downwards forwards in front of the vagina as in b b 1. Prevention of this demands support in the opposite direction similar to that in quadrupeds. A ligamentous sling around the anterior aspect of the bladder neck exerting a such postero-cranial traction does exist. Zachann (24) describes 2 fibro-elastic nodes intimately connected with the antero-lateral parts of the bladder neck. Hutch (17) describes fibres between these 2 nodes crossing the anterior aspect of the bladder neck as the precervical arc. Olesen & Grau (18) have confirmed these findings and have shown that each node continues posteriorly in a strong fibro-elastic ligament the arcus tendineus fasciae pelvis that crosses the levator muscle and tapers in the levator fascia near the sciatic notch.

Fig 1a shows a normal bladder. It is clearly seen how the bladder neck is pulled by a sling in postero-cranial direction whereby the anterior part of the bladder swells out in front of the suspension of the bladder base its anterior flat part. If this suspension loosens the bladder neck will slide downwards forwards. Secondly the ligamentous connections from the symphysis to the bladder neck the pubo-vesical ligaments therefore no longer will be taught. The latter is a common finding in stress urinary incontinence described by Krantz (14) and Zachann (24).

Variations in urethral calibre were common and variations in uro-flow in each individual during the urodynamic examination were typical. Flows were seen to vary from subnormal to high in the same patient. Twelve patients constantly presented high flows and fairly low pressures. Nine of these patients were under 50 years of age. Palm (20) describes this pattern as decreased urethral resistance. The more common pattern however was one of low or varying flows. These variations in flow rate were independent of intravesical pressure changes and therefore must be due to variations in urethral muscular tone. The varying but mostly incomplete relaxation of urethral muscular tone during micturition might hypothetically be explained

as a consequence of an increased urethral closing pressure during reservoir function. The explanation for an increased urethral closing pressure might be that when the closing of the internal urethral orifice is insufficient the remainder of the posterior urethra tries to maintain continence by increasing the muscular tone.

CONCLUSION

On the basis of bladder and vaginal morphology we have described a pathologic entity named as bladder base insufficiency. With the bladder filled but resting the bladder base is pointed and dislocated antero-distally in comparison with the normal bladder neck. The vagina is in a normal position and a gynecological examination will not therefore reveal the abnormality.

Female urinary continence depends on two factors. The urethral closing pressure created by urethral muscular tone and as the second factor adequate suspension of the bladder neck. In b b 1 the latter factor is deficient. Normally the bladder is supported by two slingformed structures. The levator muscles form a sling behind the vagina and thus support the vagina and bladder base postero-inferiorly. The other is the arcus tendineus fasciae pelvis the perurethral fibro-elastic nodes and the precervical arc that form a sling from behind around the anterior part of the bladder neck and thus support the bladder neck antero-inferiorly. In b b 1 this latter slingformed suspension or support is insufficient and the bladder neck slides downwards forwards.

Urodynamically the characteristic traits are low intravesical pressures and highly varying flows. The low pressures indicate that little effort is needed by the detrusor to open the internal urethral orifice. Thus on the other hand means that the closure of the orifice is insufficient and may well account for the complaint of these patients. Stress incontinence.

REFERENCES

- 1 Ala Ketola L. Roentgen diagnosis of female stress urinary incontinence. *Acta Obstet Gynecol Scand Supplement 23* 1 1973.
- 2 Backman K. A. von Garrehts B. & Sundblad R. Micturition in normal women. Studies of pressure and flow. *Acta Chir Scand* 132 403 1966.
- 3 Ball T. L. Douglas R. G. & F.

L. To

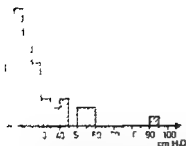


Fig 5 Bladder base insufficiency Maximum flow rates 19.3 ± 8.9 ml/sec 110 cases

41 Ob Gyn 11: 256 (1993)

Multi para s
1-2 para s
>2 para s

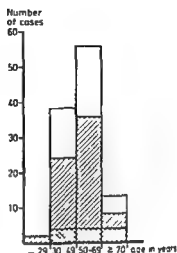


Fig 6 Bladder base insufficiency Age and parity 110 cases

The findings of numerous previous authors (2-4) Hodgekinson (9-10) and Alajouanine (11) are in accordance with this literature. We found the normal position and course of the urethra in accordance with Green's type I or a type II of the bladder during micturition which corresponds with Green's type II (7). We did not measure the posterior urethro-vesical angle because we have found it to vary from 100 to 180 in continent women. In babies, however, the downward and forward sliding of the bladder base in front of the altered vagina necessarily tends to increase the angle which is in accordance with the original observation made by Jeffcoate & Roberts (13).

Furthermore we agree with Béthoux et al (5) who on the basis of colpocystograms describe 2 bladder suspension defects: type A that leaves the

vagina unaffected and type P that produces a bulge of the anterior vaginal wall but no abnormal vaginal position. Lazarevski et al (19) confirm these findings and conclude that the 2 types A and P correspond to Green's types I and II.

The radiological signs suggest weak detrusor function in babies. This is in accordance with the urodynamic finding of rather a low opening pressure and detrusor contraction pressure and indicates that little effort is needed for the active opening of the bladder neck. On the other hand this may also account for the major symptom of stress incontinence. The bladder neck has reduced resistance to passive opening during increases in abdominal pressure.

Why is the closing of the internal urethral orifice insufficient? Opening of the orifice is a true function by detrusor contraction. Closing is passive as no distinct circular sphincter exists as shown in cross sections by Krantz (14) and Tanagho & Smith (15). Such a passive closing mechanism cannot be expected to possess any high resistance to opening unless properly supported from the outside.

Béthoux & Bory (4) make comparisons with quadrupeds. In these the major function of the levator muscle is to wag the tail. It has no other function as support for the pelvic organs. The suspension in the quadrupeds of the bladder is affected by the fascial layers of the pelvis and the

A NEW METHOD FOR PREGNANCY TERMINATION IN POLYHYDRAMNIOS

Y Beyth and M Laufer

From the Department of Obstetrics & Gynecology, Hadassah University Hospital,
Jerusalem, Israel

We wish to describe a new and safe technique for termination of anencephalic pregnancy associated with polyhydramnios which circumvents the complications of acute reduction of amniotic fluid volume in such pregnancies.

1850 ml of amniotic fluid were removed and 600 ml of Urevert were instilled; the final intraamniotic concentration of urea achieved was 5.7 g/l. Five hours after the end of the procedure, uterine contractions started and five and a half hours later the patient delivered an anencephalic male fetus. The delivery was uneventful and the patient was discharged after 3 days.

CASE REPORT

A 40-year-old gravida 7 para 6 was referred to the Ultrasound Clinic in the seventh month of her pregnancy because of severe abdominal discomfort, a large for date uterus with suspicion of twins. Ultrasonic examination revealed a term size uterus, polyhydramnios and an anencephalic fetus. The diagnosis and its implications were explained to the patient. Owing to emotional response as well as the serious discomfort occasioned by the abdominal distention, termination of pregnancy was considered to be mandatory. Due to special problems which are involved in this procedure in a grandmultipara, a variation of the usual technique of instillation of hypertonic solution was employed. An intraamniotic exchange transfusion with hypertonic urea solution (Urevert) was performed, resulting in a high concentration of urea in the amniotic fluid (Table 1). This procedure avoids acute and gross reduction of the amniotic space volume. The timing of the procedure and the volumes exchanged are indicated in the table.

DISCUSSION

Controversy exists about the management of an anencephalic pregnancy with polyhydramnios diagnosed in the third trimester. Those advocating a conservative policy point to placental abruption and uterine dysfunction as complications of the large reduction of amniotic fluid volume by surgical rupture of the membranes. Similarly, instillation of hypertonic solution to obtain high concentrations in the amniotic space requires removal of a large volume of amniotic fluid in polyhydramnios with the same risk as in surgical rupture of membranes. The risk of uterine rupture is particularly increased in grand multiparity and the use of prostaglandins is probably contraindicated in this situation.

Table 1. Exchange transfusion technique as used in the reported patient

Timing (minutes)	Procedure	Volume (ml)	Urea concentration g
0-5	Removal of amniotic fluid	800	
6-15	Instillation of hypertonic urea	600	
16-25	Intermission		
26-30	Removal of amniotic fluid	800	
31-40	Instillation of hypertonic urea	700	
41-50	Intermission		
51-55	Removal of amniotic fluid	750	1.1
56-60	Instillation of hypertonic urea	600	
61-70	Intermission		
	Removal of amniotic fluid	9	5.7

The intermissions represent periods during which fluid was neither removed nor instilled; the purpose of which was to allow equilibration.

On the other hand lack of intervention is associated with serious discomfort of polyhydramnios as well as grave emotional problems. Moreover an expectant policy in itself may lead to the complications mentioned above when premature rupture of membranes occurs.

We have employed a modified technique of instillation of hypertonic solution which circumvents the problem of dilution by the large volume of amniotic fluid in hydramnios and prevents acute reduction of amniotic fluid volume with its attendant complica-

tions as mentioned above. As described in the case report the technique of "exchange transfusion" of amniotic fluid was successful in achieving a high concentration of urea and with prompt initiation and conclusion of labor.

Submitted for publication Jan. 17, 1978

Yoram Bejth, M.D.
Dept. of Obstetrics and Gynecology
Hadassah University Hospital
Jerusalem, Israel

SHORT COMMUNICATION

SERUM VITAMIN B₆ IN PURE PREGNANCY DEPRESSION

M O Pulkkinen J Salminen and S Virtanen

From the Departments of Obstetrics and Gynecology Sero-Bacteriology and Psychiatry University of Turku Turku Finland

High levels of steroids during pregnancy may change brain metabolism and cause depression. Estrogens increase the need for pyridoxal phosphate and its deficiency is related to low brain 5-hydroxytryptamin which in turn is linked to depression (1).

Vitamin B₆ depletion may occur during pregnancy in different ways. The ability of the fetal compartment to concentrate vitamin B₆ is high (3) as a result of more binding sites for pyridoxal phosphate induced by steroids. Increased maternal corticosteroids induce vitamin B₆ requiring enzymes resulting in an increased binding in the mother. Furthermore estrogen conjugates are known to compete with pyridoxal phosphate (5, 7).

The symptoms of steroid induced depression are characterized by pessimism, dissatisfaction, crying and tension like in pure pregnancy depression. Sleep disturbances and appetite disorders often associated with endogenous and reactive depression

do not belong to this clinical picture (4). This study was designed to explore if pure pregnancy depression can be vitamin B₆-dependent.

15 patients 24±0.7 years old (mean ± S.E.) gravida 2±0.3 para 1±0.2 were 21±6 weeks pregnant and depressed for 7±7 weeks. The patients were carefully selected for this study by a psychiatrist (J. S.) excluding other forms (endogenous, reactive) of depression and patients with history of earlier clinical depression. The clinical picture of pure pregnancy depression in this study was characterized by pessimism, crying, tension and fatigue with no sleep disturbances or appetite disorders. The depth of depression was measured using the Beck Depression Inventory (BDI) (2). Initial BDI was 17±4 (7-23 included only).

Serum B₆ levels were analyzed microbiologically with the *Saccharomyces carlsbergensis* ATCC 9080 strain (6).

To check the method 7 patients were treated with 70 mg of vitamin B₆ (Heksavit®) twice a day for one week. This treatment resulted in an increase of serum concentration from 44±5.2 ng/ml to 210±32 ng/ml. Low serum B₆ reflected a deep depression ($r=0.56$, $p=0.025$, $y=-6X+97$ Fig. 1).

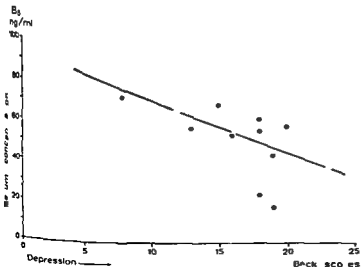


Fig. 1 Correlation between the depth of pure pregnancy depression (Beck Depression Inventory) and serum B₆ levels (microbiological method).

The present study by demonstrating a correlation between the depth of pregnancy depression and serum B₆ levels suggests that the biochemical changes (steroids → vitamin B₆) may be a cause rather than a consequence of pregnancy depression. The similarity of the clinical features of pregnancy depression and depression in users of oral contraceptives (pessimism, crying and tension with no sleep disturbances or appetite disorders) also supports the concept that pure pregnancy depression may be steroid induced.

ACKNOWLEDGEMENTS

This work was supported by the Finnish Gynecological Research Foundation. The authors are indebted to Medica Oy for drug support and for technical help to Miss Marja Ailio (vitamin assays), Mrs Arja Koivumäki (translation), Mrs Marita Väiste (typing).

REFERENCES

1 Adams P W, Rose D P, Folkard J, Wynn V, Seed M & Strong R. Effect of pyridoxine hydro-

chloride (vitamin B₆) upon depression associated with oral contraception. *Lancet* 1: 897-903 (1973).
2 Beck A T, Ward C H, Mendelson H, Mock J & Erbaugh J. An inventory for measuring depression. *Arch Gen Psych* 4: 561-571 (1961).
3 Frank O, Wahlbrocht G, Thomson A, Karsnetzky H, Kubek Z & Baker H. Placental transfer: fetal retention of some vitamins. *Am J Clin Nutr* 23: 662-663 (1970).
4 Herzberg B N, Johnson A L & Brown S. Depressive symptoms and oral contraceptives. *Br Med J* 4: 142-145 (1970).
5 Mason M, Ford J & Wuh H L C. Effects of steroid and nonsteroid metabolites on enzyme covalent modification and pyridoxal phosphate binding. *Ann NY Acad Sci* 166: 170-183 (1969).
6 Microbiological assay of vitamins and aminoacids pp 1-48-49. *Disco*, Detroit (1969).
7 Pulkkinen M O & Willman A. In vitro regulation of human placental aspartate aminotransferase by sulfate esters of estrogens. *Steroids* 31: 51-59 (1966).

Submitted for publication April 9 1977

Martti Pulkkinen
Dept of Obstetrics and Gynecology
University of Turku
SF-20520 Turku 57
Finland

CASE REPORTS

THE ETHANOL GELATION TEST IN PREGNANCY

C Eika H Arnesen and H C Godal

*From Hematological Research Laboratory and Department IX
Ullevål University Hospital Oslo Norway*

Abstract Coagulation studies in 55 healthy pregnant women indicated that in spite of high coagulation activity a positive ethanol gelation test for fibrin in plasma is not a normal feature of pregnancy. The four case reports presented stress the value of the ethanol test in monitoring treatment of thromboembolic disease in pregnancy.

During pregnancy increased levels of coagulation factors and reduced fibrinolytic activity are regularly observed (4, 3). This hypercoagulable state is accompanied by increased risk of thromboembolic disease (11, 1).

The ethanol gelation test (EGT) (7) indicates the presence of soluble fibrin in plasma and a positive test is often found during intravascular coagulation and other thromboembolic disease (9).

At Department IX Ullevål Hospital we have during recent years treated 4 pregnant patients with different manifestations of thromboembolic disease, all of them having a positive EGT. The positive test, however, might reflect the hypercoagulable state of pregnancy and as such be a normal finding in pregnancy. In order to clarify the significance of a positive EGT in pregnancy a group of healthy pregnant women were examined.

MATERIALS AND METHODS

Fifty-five healthy pregnant women were examined. None of them had present or previous history of thromboembolic disease. Nine were in the first trimester, 19 in the second and 27 in the third trimester. The analyses performed and references for the methods are given in Table I.

RESULTS

The results of the coagulation studies are given in Table I. The increased coagulation potential during pregnancy was reflected by high Normotest/

Thrombotest values and a slight shortening of the prothrombin time. A slight increase in antithrombin III was observed. The most marked change was the increase in fibrinogen during late pregnancy.

In all the healthy pregnant women studied only one had a positive EGT (3rd trimester). This woman later developed a deep venous thrombosis.

CASE REPORTS

Case I

Born 1948. Pregnant with expected delivery March 15 1972. In November 1971 admitted to hospital with chest pains without previous symptoms of deep venous thrombosis. X-ray examination showed right-sided pleural fluid. The diagnosis of pulmonary embolism was made and the patient treated with heparin intravenously at first and later subcutaneously. Initially the ethanol test was positive. During i.v. heparin treatment it became negative. After changing to subcutaneous heparin the test again became positive and intravenous treatment was reestablished. On reverting to subcutaneous heparin the dose had to be fixed at 37 500 units/day to prevent a positive ethanol test. On March 19th 1972 she had a normal delivery and the anticoagulant regimen was changed to warfarin. This was ended in June. The ethanol test remained negative after delivery. No further thromboembolic complications have occurred.

Case II

Born 1948. Admitted to hospital for abruptio placenta on June 11th 1972 and was delivered of a girl by caesarean section. Profuse bleeding from the vagina occurred (non-clotting blood). Shortly after delivery thrombocytes were $25 \times 10^9/l$, fibrinogen 0.97 g/l and the ethanol test was positive. The patient was treated with blood transfusion 500 ml $\times 6$ during 4 hours. In addition she received Trasylol[®] and purified fibrinogen 2 g. Four hours after delivery thrombocytes were $11 \times 10^9/l$ and fibrinogen 1.5 g/l. The ethanol test was still positive. Eleven hours after delivery the thrombocytes had increased to $11.5 \times 10^9/l$. Fibrinogen had increased to 2 g/l and the ethanol test had become negative. The next day thrombocytes were unchanged, fibrinogen was 3.65 g/l and the ethanol test negative. Clinically the patient improved rapidly and completely.

Table 1 Coagulation values in 55 healthy pregnant women

	Ref	Normal values	Values in 55 pregnant women					
			1st trimester (9)		2nd trimester (19)		3rd trimester (27)	
			Mean	Range	Mean	Range	Mean	Range
Normotest (%)		65-150	97	87-130	122	97-143	125	80-150
Thrombotest (%)		50						
Thromboplastin time (sec)	(9)	13-17	15.5	13-18	14.2	13-16	14.1	11-16
Partial thromboplastin time (sec)	(10)	60-85	67.5	56-92	69.0	57-97	68.0	41-81
Antithrombin III (plasma) (%)	(6)	100-150	121	93-158	123	76-144	113	93-141
Fibrinogen (mg%)	(2)	170-400	243	147-377	25	146-387	364	66-671
Ethanol gelation test	(7)	neg	neg		neg		no neg	1 pos

Case III

Born 1939. Pregnant with expected delivery August 24th 1972. No previous thromboembolic disease when admitted to hospital January 26th with symptoms of deep venous thrombosis in the left leg and pulmonary embolism. Coagulation studies revealed signs of intravascular coagulation with consumption of thrombocytes ($490-125 \times 10^9/l$) and of fibrinogen ($6.3-2.35 \text{ g/l}$). NT/TT-discrepancy ($170\%/68\%$) and a positive ethanol test. She was treated with heparin 30 000 IU/day by continuous intravenous infusion. The coagulation factors including the ethanol test returned to normal. Several attempts to discontinue heparin during the first weeks were followed by reappearance of a positive ethanol test, a drop in the fibrinogen and thrombocyte values as well as increased pain and swelling of her left leg. After 6 weeks heparin was discontinued with maintenance of a negative ethanol test. During delivery heparin was given subcutaneously 25 000 IU/day divided into two doses. As the positive ethanol test accompanied by pain in her left leg, recurred the day after delivery the subcutaneous heparin treatment was continued for 5 days until a negative ethanol test was obtained. Thereafter warfarin was given for 6 months. Later blood samples showed no signs of hypercoagulability but phlebography 3 months after delivery showed sequelae of thrombosis in the left femoral vein.

Case IV

Born 1955. Pregnant with expected delivery May 29th 1973. Admitted to hospital March 3rd with clinical signs of deep venous thrombosis in the left leg. Coagulation studies revealed slight intravascular coagulation with positive ethanol test but without obvious signs of consumption of fibrinogen or thrombocytes. Treatment with intravenous infusions of heparin ($40-60 000 \text{ IU/day}$) was started. After 10 days heparin was administered subcutaneously 75 000 IU/day divided into two doses. The ethanol test became negative after 2 weeks. The patient left hospital after 18 days without medication. Three days after discharge the ethanol test was again positive and treatment with heparin was reinstituted subcutaneously 50 000 IU/day divided into two doses. The ethanol test

remained slightly positive during the rest of pregnancy but was negative the day after delivery. On the first day postpartum heparin was stopped and treatment with warfarin was started and continued for 3 months. Later blood samples showed no signs of hypercoagulability but phlebography 3 months after delivery revealed sequelae of thrombosis in the left femoral vein.

COMMENTS

In diseases associated with a high incidence of venous thrombosis hypercoagulability as reflected by high levels of fibrinogen and other coagulation factors is regularly found. In addition a positive ethanol test for fibrinemia is often observed (9). In the present study previous reports on blood changes during pregnancy were confirmed (4). In spite of this hypercoagulable state a positive ethanol test is most uncommon in normal pregnancy. Thus the only woman with a positive test in the present material developed a deep vein thrombosis the day after delivery. It seems therefore that a positive ethanol test is clinically significant even in late pregnancy and indicates increased risk of thromboembolic complications. It is significant that increased serum levels of fibrin degradation products in pregnancies have been shown to be associated with a marked increase in postpartum thromboembolic complications (8).

In addition to its diagnostic value the ethanol test may prove useful in monitoring treatment and in detecting recurrences as shown in the above case reports. It should be stressed however that the occurrence of a positive ethanol test does not always necessitate the use of anticoagulants as it was

found in case II that intravascular coagulation may be a self limiting state when the triggering factor is removed

REFERENCES

- 1 Aaro L A & Juergens J L Thrombophlebitis as associated with pregnancy *Am J Obstet Gynecol* 109 1178 1971
- 2 Arnesen H & Godal H C Simple routine method for fibrinogen determination *Tidsskr Norske Lægeforen* 24 1576 1977
- 3 Biezenski J J & Moore H C Fibrinolysis in normal pregnancy *J Clin Pat* 11 306 1958
- 4 Bonnar J McNicol G P & Douglas A B Coagulation defects in obstetrics *In Modern Trends in Obstetrics* (ed R J Kellar) Butterworths London 1969
- 5 Borchersink C F & Waaler B A The secondary bleeding time A new method for the differentiation of hemorrhagic diseases *Acta Med Scand*
- 6 Fagerhol M & Abildgaard U Immunological studies of antithrombin III *Scand J Haematol* 7 10 1970
- 7 Godal H C & Abildgaard U Gelation of soluble fibrin in plasma by ethanol *Scand J Haematol* 3 347 1966
- 8 Hedner U & Åstedt B Studies on fibrinolytic inhibitors during pregnancy *Acta Obstet Gynecol Scand* 50 99 1971
- 9 Kierulf P & Godal H C Fibrinemia in medical patients screened by the ethanol gelation test *Acta Med Scand* 189 37 1971
- 10 Proctor R R & Rapaport S I The partial thromboplastin time with kaolin A simple screening test for first stage plasma clotting factor deficiencies *Am J Clin Pathol* 36 717 1961
- 11 Villasanta U Thromboembolic disease in pregnancy *Am J Obstet Gynecol* 93 147 1965

Submitted for publication June 16 1977

Clas Eika M D
Avdeling IX
Ullevål Sykehus
Oslo
Norway

A PREGNANCY WITH A HYDATIDIFORM MOLE THYREOTOXICOSIS AND LIVE BORN INFANT

Poul Ladehoff and Andrzej Maruszczak

From the Department of Obstetrics and Gynaecology Odense University Hospital and the Department of Obstetrics and Gynaecology Sønderborg Hospital Denmark

Abstract A 6-year-old woman pregnant for the 3rd time was admitted in the 2nd trimester with bleeding and slight signs of thyreotoxicosis. The free thyroxin, total thyroxin and TRH test confirmed the diagnosis. The patient was delivered 8 weeks before term of a living boy of 1680 g. Immediately after the child a 300 g normal placenta was born followed by 650 g of molar tissue.

Four weeks after birth and evacuation of the molar tissue the patient was euthyroid with normal thyroid parameters. It is presumed to have been a twin pregnancy in which the part transformed to molar tissue has secreted a thyroid stimulating factor.

The majority of cases of hydatidiform mole present with symptoms in the 16th to 24th week and the course of the disease has been described as being similar to abortion (1, 2, 3).

The concomitant occurrence of a mole and a normally developed foetus is a rare phenomenon. A survey of the literature (4, 10, 11) showed a frequency of approximately 1/14 000 births in areas in which the incidence of hydatidiform mole is approximately 1/700. In other words the concomitant occurrence of a mole and foetus is approximately 1/14 of all cases of a mole.

The delivery of a living infant in connection with the changes brought about by a mole occurs extremely rarely. A survey from Australia covering 6 years showed 29 births of fetuses together with moles but of these only 6 were live born. 5 were twin pregnancies, 3 dizygotic and 2 monozygotic. There was only one case of a single pregnancy (1, 2).

A considerable increase in thyroid function with or without clinical signs of hyperthyroidism occurs in connection with hydatidiform mole (6, 7, 8, 9, 12, 13) and with chorocarcinoma (6). The hyperthyroidism disappears and the thyroid function returns to normal following chemotherapeutic treatment of the chorocarcinoma or evacuation of the mole (6, 7, 8, 9, 12).

A specific thyroid stimulating factor that differs from long acting thyroid stimulator has been found in molar tissue and has been termed molar thyroid stimulating factor (5, 8). However Tojo et al (12, 13) are of the opinion that the thyroid stimulating factor in molar tissue is identical to that found in normal human placenta but that it is merely secreted in abnormal amounts by the molar tissue. It has been stated that human chorionic gonadotropin can induce thyreotoxicosis (7).

A case like the present one with a living infant mole and hyperthyroidism has not been described before. We therefore consider it of interest to report the case.

CASE REPORT

(M. L. 220750) A previously healthy 26-year-old woman gravida 3 para 3 was admitted during the third pregnancy from the 12th to 14th week with vague abdominal pains and vaginal bleeding. The uterus was found to be slightly larger than indicated by the menostasis and heart sounds were heard with the aid of a dopstone. The symptoms disappeared spontaneously. She suffered from nausea and periodic vomiting during the whole of the first and part of the second trimester. The patient was readmitted from the 24th week owing to vomiting, loss of weight of 5 kg and spotting. She was found to be restless with a warm and sweaty skin as well as having a fine tremor of the hands. No exophthalmos or goiter was present. The thyroid parameters: T_4 -test 63-66% corresponding to a normal pregnancy, free thyroxin 96.6-77.5 pmol/l (70.0-36.1), total thyroxin 324-231 nmol/l (66-139). A TRH test showed no rise in TSH values 11.2-8.1-9.0 μ U/ml. The results of this test together with the increased values of free thyroxin indicated that the patient suffered from thyreotoxicosis. A placenta scintigraphy with Tc^{99m} alb was carried out owing to the continued spotting. This showed strange large areas of reduced activity in the placental region and a deep placental scar (Fig. 1). These areas were interpreted as being infarctions. The oestriol excretion in the urine showed a normal rise. The membranes ruptured in the 37th week of pregnancy.

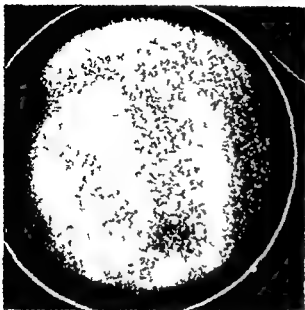


Fig 1 Placenta scintigraphy

and the patient was spontaneously delivered of a living male child without complications. The birth weight was 1680 g. The placenta was delivered together with large amounts of molar tissue immediately after the child. Considerable amounts of molar tissue were removed manually and with a blunt curette. The placenta weighed 300 g and had no septum. The total weight of the molar tissue was 650 g. The patient was discharged from hospital 5 days after delivery. Histological examination: 1) underdeveloped placenta. 2) hydatidiform mole. A control examination 4 weeks after birth showed that the patient was euthyroid with normal thyroid parameters: T_4 test 86 pmol/l, free thyroxine 19 pmol/l, total thyroxine 85 nmol/l, hCG negative. The ovaries were enlarged (5.6 cm in diameter). At a control examination 6 weeks after birth the ovaries were normal. The child was discharged 5 weeks after birth with a weight of 7400 g and no signs of any metabolic disease.

DISCUSSION

The etiology of hydatidiform mole is unknown but it has been suggested that it is primarily a defect of the ovum (13).

In our case there could have been a twin pregnancy. We have not found any septum and no chromosome analysis was carried out on the molar tissue so we cannot draw any definite conclusions about this.

Unlike others (6, 7, 8, 12, 13) we were not in a position to isolate the thyroid stimulating factor. But the various thyroid parameter which clearly demonstrated that the patient was thyrotoxic were completely normal 4 weeks after removal of

the molar tissue. This would suggest that the mole was the cause of the condition. This is in agreement with others (7, 12) who found complete normalization of the parameters within the first week following evacuation. Our patient had only a few clinical signs of thyrotoxicosis during the first part of the hospitalization, signs which could easily have been overlooked or interpreted as normal problems with the pregnancy. Such a course with slight to moderate symptoms is the most frequent (8, 14). But this condition can also develop into a life threatening state with severe hyperthyroidism and pulmonary oedema which disappear promptly after evacuation of the mole (6, 9).

REFERENCES

1. Berscher N A. Hydatidiform mole with coexistent foetus. *J Obstet Gynecol Br Comm* 68: 731 1961.
2. Berscher N A et al. Significance of chromosomal terms in cases of hydatidiform mole with an associated fetus. *Am J Obstet Gynecol* 100: 776 1964.
3. Benirschke H & Driscoll S G. The pathology of the human placenta. Springer Verlag New York 1969.
4. De George F V. Hydatidiform moles in other pregnancies of mothers of twins. *Am J Obstet Gynecol* 108: 769 1970.
5. Hershman J M & Higgins H P & Starnes W J. Differences between thyroid stimulator in hydatidiform mole and human chorionic thyrotropin. *Metabolism* 19: 735 1970.
6. Hershman J M & Higgins H P. Hydatidiform mole—a cause of clinical hyperthyroidism. *N Engl J Med* 289: 573 1973.
7. Higgins H P et al. The thyrotrophic activity of hydatidiform mole. *Ann Intern Med* 24: 107 1974.
8. Galton V N et al. Alterations in thyroid hormone economy in patients with hydatidiform mole. *J Clin Invest* 50: 1345 1972.
9. Kristoffersen K & Jørgensen F S. A case of hydatidiform mole with severe pre-eclampsia and severe disturbances in thyroid function. *Acta Obstet Gynecol Scand* 49: 119 1970.
10. Logan B J. Occurrence of a hydatidiform mole twin pregnancy. *Am J Obstet Gynecol* 23: 911 1967.
11. Sitaranta A. Twin pregnancy with a normal viable fetus and a hydatidiform mole associated with a normal viable fetus. *J Obstet Gynecol Br Comm* 67: 101 1960.
12. Tojo S et al. Human chorionic TSH (hTSH) hCG during normal or molar pregnancy. *Endocrinol Jpn* 20: 405 1973.
13. Tojo S et al. hTCT and thyroid function in molar pregnancy. *Acta Obstet Gynecol Scand* 55: 141 1976.

Submitted for publication June 6 1977

Poul Ladehoff
Karolinevej 6
4400 Ålbøllert
Denmark

MYOMETRIAL ACTIVITY AND ENDOMETRIAL BLOOD FLOW IN AN ECTOPIC PREGNANCY

Mats Åkerlund

*From the Department of Obstetrics and Gynaecology, University Hospital
Lund, Sweden*

Abstract Myometrial activity and endometrial blood flow were recorded in a patient with uterine haemorrhage and lower abdominal pain who turned out to have a tubal pregnancy. The uterine contractions had an amplitude sometimes exceeding 400 mmHg and a duration up to 3.5 min. Thus strong uterine contractions can appear during pregnancy without the presence of a gestational sac within the uterus. This is contrary to the assumption that processes in the foetal membranes initiate the sequence of events leading to abortion or labour.

There is an increasing amount of evidence suggesting that degenerative changes in the decidua initiate a synthesis and liberation of prostaglandins (PG) and that these PGs induce labour and abortion (cf 5). Recently Schwarz, Schultz, McDonald and Johnston (10) suggested that an obligatory precursor in the synthesis of PGE_2 and PGE_2 arachidonic acid is released from the foetal membranes and diffuses to the decidua. Gustavii (7) on the other hand proposed that PG precursor acids are derived from phospholipids of cellular membranes in decidual cells. An indirect way to study the importance of the foetal membranes as a source of PG precursors appeared during a recent study on primary dysmenorrhoea (1). Accidentally intrauterine pressure and endometrial blood flow was recorded in a woman with uterine haemorrhage and lower abdominal pain who turned out to have a tubal pregnancy.

CASE REPORT

The woman investigated was a 33 year old nurse who with the exception of a moderate primary dysmenorrhoea had previously been healthy. She volunteered for a study on primary dysmenorrhoea and was well informed of the experimental procedure. She had never been pregnant previously but had finished the use of contraceptive pills 6 months earlier and since then she had not menstruated.

Conventional pregnancy tests (Pregnosticon Planotest Pharmacia AB, Sweden) taken both 20 and 7 days before the recording session had been negative. The patient denied any symptoms of pregnancy such as breast engorgement and morning nausea. She was registered during what was assumed to be an ordinary menstruation. The investigation started 48 h after the onset of a spotting vaginal bleeding and 5 h after the onset of a colicky lower abdominal pain accompanied by general symptoms of nausea and chill. In a blood sample obtained at the beginning of the recording session the plasma concentration of progesterone was 0.40 ng/ml and of oestradiol 70 pg/ml determined by radioimmunoassay. Myometrial activity was recorded as changes in intrauterine pressure (3) and local decidual blood flow was registered by a technique based on measuring thermoconduction from a heated thermistor to blood flow in the surrounding tissue (2).

The original recording of intrauterine pressure and blood flow in the patient one hour after the start of the recording is shown in Fig. 1. The patient had regular and well-defined uterine contractions with multiple peaks. The contractions varied in amplitude between 700 and 425 mmHg in duration between 2 and 3.5 min and in frequency between 2 and 4 contractions per 15 min. After every contraction there was a period of uterine relaxation the basal tone being about 25 mmHg. The local endometrial blood flow decreased markedly during each contraction but resumed its previous level in between.

During the recording the patient continuously reported all changes in character and intensity of her pain. This was noted on the recording curve which was not visible to her. It was found that the colicky pain appeared in periods which corresponded to the periods of uterine contraction and decrease in blood flow. Between these periods the patient felt practically no pain.

As a part of the intended study on dysmenorrhoea the selective β_2 -receptor stimulator terbutaline (Bricanyl® Draco, Sweden) was given after 2 h and 15 min of recording as a single intravenous injection of 0.25 mg during 3 min. The drug effectively inhibited the uterine contractile activity and only a few contractions of a small amplitude appeared during 45 min of recording after the injection. The local endometrial blood flow remained fairly constant after the administration of terbutaline except for decreases in flow occurring in parallel with the remaining

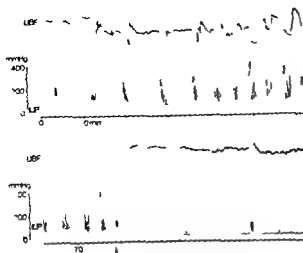


Fig 1 Local endometrial blood flow (UBF) intrauterine pressure (IUP) and the effect of terbutaline (0.25 mg i.v. injection indicated by arrow) in a woman with lower abdominal pain and vaginal bleeding due to an ectopic pregnancy

myometrial contractions. After the injection the patient reported a total relief of her low abdominal pain and when it returned after about 2 h it was much less pronounced.

During the days after the recording the woman had repeated periods of slight vaginal bleeding or spotting and also periods of moderate colicky pain. Because of these symptoms she underwent laparoscopy and laparotomy 10 days after the recording. An ampullary pregnancy in her right Fallopian tube was found and expelled. The ovum had a diameter of about 9 mm. At histopathological examination chorion villi but no embryonal structures were found.

DISCUSSION

The plasma concentrations of oestradiol and progesterone at the recording corresponded to normal values in nonpregnant women during the first menstrual days (9). Consequently it can be assumed that the spotting vaginal bleeding in the present woman was the result of withdrawal of hormonal stimulation of the decidua with consequent degeneration and shedding. According to the hypothesis of Gustavii and others (cf. 6) this decidual degeneration may have initiated the uterine activity.

The uterine activity in the present case resembled recordings from intrauterine pregnancies (e.g. 4) in as much as the contractions were well-defined regular and had intervening periods of complete uterine relaxation. There were however important differences, the most remarkable finding in the ectopic pregnancy being the extremely high am-

plitude and long duration of the contractions. A comparison of amplitudes as an index of myometrial activity in a uterus containing a gestational sac with one that does not is however difficult since according to the law of Laplace the muscular work required to produce a certain intrauterine pressure increases with the squared radius of the intrauterine cavity. In the nonpregnant uterus such high pressures were not recorded as seen in the present case, not even in severe primary dysmenorrhoea (1) or after expulsion of an IUD (4).

The findings in this case of ectopic pregnancy suggest that the presence of an intrauterine gestational sac is not of importance for the induction of uterine contractions at decidual degeneration. This supports the suggestion of Gustavii (7) that PG precursor acids can originate from within the decidual cells. The extremely strong and long lasting uterine contractions found in the present woman are rarely seen in physiological conditions. It is tempting to speculate whether this activity might be due to the lack of an inhibitory function of an intrauterine pregnancy. Such a concept would be in accordance with the findings of Heirse et al. (8) who in tissue from early pregnant human uterus demonstrated high concentrations of PG degrading enzymes in placenta and foetal membranes but only small concentrations of these enzymes in myometrium and decidua.

REFERENCES

- 1 Åkerlund M, Andersson A E & Ingemarsson I. Effects of terbutaline on myometrial activity, uterine blood flow and lower abdominal pain in women with primary dysmenorrhoea. *Br J Obstet Gynaecol* 83: 673, 1976.
- 2 Åkerlund M, Bengtsson L Ph & Carrier A M. A technique for monitoring endometrial or decidual blood flow with an intra uterine thermistor probe. *Acta Obstet Gynecol Scand* 54: 46, 1975.
- 3 Åkerlund M, Bengtsson L Ph & Lillemor L. Recording of myometrial activity in the non-pregnant human uterus by a micro-transducer catheter. *Acta Obstet Gynecol Scand* 57: 479, 1978.
- 4 Åkerlund M & Laudanski T. Induction of uterine activity in early therapeutic abortion by a large dose vasopressin analogue. *Br J Obstet Gynaecol* (in press).
- 5 Bengtsson L Ph & Moawad A H. The effect of the Lysine loop on human myometrial activity. *Am J Obstet Gynecol* 58: 957, 1967.
- 6 Gustavii B. Physiology of initiation of parturition in humans. *Neonatology—Nuclear Medicine vol 1*. 14th International Congress of Pediatrics 1977.

- 17 Editorial Medica Panamericana Buenos Aires 1974
- 18 Gustav B Sweeping of the fetal membranes by a physiologic saline solution Effect on decidual cells *Am J Obstet Gynecol* 120 531 1974
- 19 Keirse M J N C Williamson J G & Turnbull A O Metabolism of prostaglandin F_2 within the human uterus in early pregnancy *Br J Obstet Gynaecol* 87 14 1975
- 20 Mishell D R Jr Nakamura R M Crosgnani P J Stone S Kharna K Nagata Y & Thorneycraft I H Serum gonadotropin and steroid patterns during the normal menstrual cycle *Am J Obstet Gynecol* 111 60 1971
- 21 Schwarz H F Schultze F M MacDonald P C & Johnston J M Initiation of human parturition *III Obstet Gynecol* 46 564 1975

Submitted for publication Oct 26 1977

Mats Åkerlund
Department of Obstetrics and Gynecology
University Hospital
Lund
Sweden

ANENCEPHALY AND CLOMIPHENE INDUCED PREGNANCY

Y Biale, H Leventhal, M Altaras and N Ben Aderet

From the Department of Obstetrics and Gynecology, A. The Soroka Medical Center, Beersheba, Israel

Cloimphene citrate is used extensively today to induce ovulation in an anovulatory female to improve fertility. It is well known that the incidence of multiple pregnancies, abortions and overstimulation syndrome in clomiphene induced pregnancies is higher than in non induced pregnancies (1). Recent publications indicate an association with hydatidiform mole in clomiphene related pregnancies (2, 3, 4). The following are two case reports of anencephalic births to mothers who had received clomiphene as an ovulatory stimulus.

CASE REPORTS

Case 1 A 37-year-old woman married 17 years with no previous pregnancy. Menarche at age 13 with regular periods. Basal body temperature usually biphasic with normal ovulatory levels of pregnandiol. Hysterosalpingography showed a normal uterus with patent tubes. Since the husband was found to be totally azospermic it was decided to perform donor artificial insemination. Due to the fact that pregnancy did not ensue 100 mg clomiphene citrate was given for a total of 5 days from the fifth day of the cycle. Under this treatment she became pregnant. The pregnancy followed a normal course until the eighth month when a diagnosis of small for dates was made. Estriol levels were low at 3000 µg/74 hours. X-ray examination of the abdomen showed an anencephalic fetus. A spontaneous delivery occurred at the end of the ninth month.

Case 2 A 6-year-old para 1 gravida 2 with irregular menstruation 30-70/4 since the age of 14 years. The first pregnancy was induced by clomiphene citrate treatment and she gave birth to a boy weighing 3800 g. The present pregnancy was also induced after the third clomiphene treatment: 100 mg per day for five days. In the 43rd week of pregnancy she was referred to the delivery room because of post maturity.

The height of the uterine fundus fitted eight months of pregnancy. There was breech presentation with closed cervical os. Fetal movements and heart beats were normal. The amniocentesis demonstrated clear amniotic fluid. Estriol excretion on two subsequent days was 4 and 4.9 µg per 74 hours. An abdominal X-ray confirmed the suspicion of an anencephalus with breech presentation.

Three days after admission she delivered an anencephalic monster with complete cranio-spinal rachischisis.

DISCUSSION

Most investigations are now agreed that there is multifactorial causation for anencephaly including genetic element with environmental factors. Drugs that stimulate ovulation have not been included in the etiological factors of anencephaly. Dayson & Kohler were the first to report anencephaly following ovulation stimulated by clomiphene in two women (5). Further sporadic case reports of anencephaly occurring with ovulation stimulation by clomiphene have appeared more recently (6, 7). These observations are compatible with Boue & Boue's recent observation of the increased frequency of chromosomal anomalies in abortions after induced ovulation (8). Because of the rare association of anencephaly with ovulation induced by clomiphene it is claimed by James (9) that the defect may be related to the underlying subfertility for which the drug was administered rather than to the drug itself. James offered some evidence that women who produce anencephalies are less fecund than other women. On reviewing the literature we consider that the possibility of a casual relationship between ovulation stimulating treatment and central nervous system abnormalities should not be dismissed lightly but much additional evidence will be needed to establish the hypothesis.

REFERENCES

1. MacGregor A H, Johnson J E & Bunde C A. Further clinical experience with clomiphene citrate. *Fertil Steril* 19: 616 1968.
2. Miles P A, Taylor H B & Hill W C. Hydatidiform mole in a clomiphene related pregnancy. *Obstet Gynecol* 37: 358 1971.
3. Schneidermann C I & Waxman B. Clomid therapy

- and subsequent hydatiform mole formation. *Obstet Gynecol* 39: 787, 1972
4. Wayntraub G., Kamar R. & Pardo Y. Hydatidiform mole after treatment with clomiphene. *Fertil Steril* 25: 904, 1974
 5. Dayson J. L. & Kohler H. H. Anencephaly and ovulation stimulation. *Lancet* 1: 1256, 1973
 6. Sandler H. Anencephaly and ovulation stimulation. *Lancet* 2: 379, 1973
 7. Barrett C. & Hakim C. Anencephaly, ovulation stimulation, subfertility and illegitimacy. *Lancet* 2: 916, 1973
 8. Boué J. G. & Boué A. Increased frequency of chromosomal anomalies in abortions after ovulation stimulation. *Lancet* 1: 679, 1973
 9. James W. M. Anencephaly, ovulation stimulation and subfertility. *Lancet* 2: 916, 1973

Submitted for publication August 7, 1977

Y. Biale
Department of Obstetrics and Gynecology
A. The Soroka Medical Center
BeerSheva
Israel

INDEX

Letters Å and A—see under A letters Ø and Ö—see under O

- Ammons K see Myers 317
- Ahlberg G and Ahlmark G The Landry-Gunlan-Barre syndrome and pregnancy 377
- Ahlmark G see Ahlberg 377
- Åkerlund M Myometrial activity and endometrial blood flow in an ectopic pregnancy 479
- Åkerlund M Bengtsson L and Ulmsten U Recording of myometrial activity in the non-pregnant human uterus by a microtransducer catheter 479
- Åm P see Thorbert 45
- Årén M see Biåle 483
- Årén H T see Maltun 191
- Andersson K E see Rud 457
- Andersson K see Wingerup 403
- Andersen H see Eika 473
- Andersen Inger Ultrastructure of human umbilical veins 53
- Andersson O Lindberg B S Nilsson B A and Johansson E D B Plasma levels of non-conjugated oestrone in high risk pregnancies 113
- Ashary C see Leiba 373
- Ashkenazi L Hoffman H J and Sternthal P M Obstetric service and perinatal mortality in Norway suppl 77
- Ås A see Mazor 273
- Beckman Gunhild Beckman L and Lofstrand T Acid and alkaline phosphatase in amniotic fluid in normal and complicated pregnancy 1
- Beckman L see Beckman 1
- Behre I see Neme 19
- Ben Aderet N see Biåle 349
- Ben Aderet N see Biåle 483
- Bengtsson L Ph see Åkerlund 429
- Berg G see Ryden 313
- Bergman B and Hedner T Antepartum administration of terbutaline and the incidence of hyaline membrane disease in preterm infants 217
- Bergman B Hedner T and Lundborg P Effects of terbutaline on the pressure-volume relationship in fetal rabbit lung 33
- Bergqvist G Holmberg G Rydner T and Vacklinova Vlasta Intrauterine death due to infection with group B streptococci 127
- Beyth Y Improved method for hystero-graphic evaluation of uterine scar 111
- Beyth Y and Laufer N A new method for pregnancy termination in polyhydramnios 469
- Biåle Y Lazer S and Ben Aderet N Fracture and chemical composition of the deposit formed on the Lippes loop after prolonged use 349
- Biåle Y Leventhal H Alteras M and Ben Aderet N Anencephaly and clomiphene induced pregnancy 483
- Bieniarz J Shah N Dmowski W P Rao R and Scornegna A Premature labor treatment with Ritodrine in multiple pregnancy with three or more fetuses 25
- Borenstein M see Dgani 385
- Brand A see Wallenburg 105
- Breitnecker G see Husslein 73
- Brenner W E see Dingfelder 35
- Brohult Astrid Brohult J and Brohult M Regression of tumour growth after administration of alkoxyglycerols 79
- Brohult J see Brohult 79
- Brohult S see Brohult 79
- Bunne G and Öbrink A Influence of pubococcygeal repair on urethral closure pressure at stress 355
- Bunne G see Öbrink 49
- Bygdeman M see Martin 141
- Carlstrom K see Stånge 289
- Cederqvist L L see Rothe 7
- Chretien F C Ultrastructure and variations of human cervical mucus during pregnancy and the menopause 337
- Christau Susanne and Klebe J G Rupture of the spleen during delivery 187
- Coats P Florensa E Youssefnejadian E and Craft I Plasma steroids in the foetal and maternal circulation at normal delivery and elective caesarean section 171
- Cominos A C see Kalpakisoglou 85
- Craft I see Coats 121
- Cullhed S Carcinoma cervicis uteri stages I and IIa suppl 75
- Czernobitsky B see Dgani 385
- Dalen N Furuhejm Minam Jacobson B and Lamke H Changes in bone mineral content in women with natural menopause during treatment with female sex hormones 435
- David M P see Deligdisch 439
- Deguchi M see Mochizuki 397
- Deligdisch Liane Yedwab G Persitz A and David M P Ultrastructural features in normal and hyperplastic postmenopausal endometrium 439
- Deligdisch Liane see Mazor 273
- Dgani R Czernobitsky B Borenstein R and Lancet M Granular cell myoblastoma of the vulva Report of 4 cases 385
- Diedrich P see Møller 41
- Digrahan A see Garam 453
- Dingfelder J R and Brenner W E The thermogenic activity of exogenous E and F prostaglandins in humans 35
- Dmowski W P see Bieniarz 25
- Dolezal A see Štulec 175
- Drabkova J see Štulec 1,5
- Edelman D A see Ragab 327
- Eika C Arnesen H and Godal H C The ethanol gelation test in pregnancy 473
- Emmerth Y Cryosurgical treatment of dysplasia and carcinoma in situ of the cervix uteri 361
- Eneroth P see Martin 141
- Eneroth M see Moberg 415

- Enksson Margareta see Stånge 289
- Erkkola R see Lammintausta 389
- Eronen M see Lammintausta 389
- Fagerlund Christina see Meink 287
- Falck Larsen J, Jacobsen B, Holm H H, Fog Peder sen J and Mantoni Margit Intrauterine injection of vitamin K before the delivery during anticoagulant therapy of the mother 227
- Fianu S Maternal mortality in Sweden 1955-1974 179
- Fianu S and Václavinková Vlasta The site of placental attachment as a factor in the etiology of breech presentation 371
- Florensa E see Couto 121
- Fog Pedersen J see Falck Larsen 227
- Frberg L G, Kullander S, Persson J P and Korsten C B On receptors for estrogens (E₂) and androgens (DHT) in human endometrial carcinoma and ovarian tumours 761
- Frack G, Johnsson J E, Landberg T and Snorra dottir M Relaparotomy in advanced ovarian carcinoma 165
- Fuchs F see Rothe 7
- Furuhjelm Minam see Dalén 435
- Fylling P see Jerve 237
- Ga de P, Teale D and Pedersen H Extremely low placental lactogen hormone (hPL) values in an otherwise uneventful pregnancy preceding delivery of a normal baby 93
- Gennert G see Persson suppl 78
- Gjonnæs H and Holten F Doxycycline (Vibramycin®) in pelvic inflammatory disease 137
- Gudal H C see Eika 473
- Greke C J and Fjer J J Bipolar cautery for laparotomy 169
- Greke C J see Persson suppl 78
- Greke C B see Persson suppl 78
- Gronan E see Ikonen 741
- Grunar M and Larsson Cohn U Massive enlargement of occluded tubes after postmenopausal treatment with natural estrogens 189
- Hansen J T see Muller 41
- Hansen J T see Muller 133
- Hansson U, Iredell L and Moberg P J Delivery complicated by myasthenia gravis and epilepsy 183
- Haram K and Digrane A Vulvovaginal candidiasis in pregnancy treated with clotrimazole 453
- Harlin J see Moberg 415
- Hartvig P see Meink 28
- Hashiba N see Nakano 793
- Hedman Anna Kristina see Václavinková 69
- Hedner T see Bergman 1
- Hedner T see Bergman 11
- Hoffman H J see Bakker suppl 77
- Holm H H see Falck Larsen
- Holmberg G see Bergqvist 1
- Holten F see Gjonnæs 13
- Honda T see Mochizuki 179
- Huilein H, Breitenacker C and Istra G Pre-malignant and malignant uterine changes in immunosuppressed renal transplant recipients 3
- Ikonen R S, Hagman F and Pärönen T Effect of fasting on blood glucose of parturient after full term infant 41
- Ingelman Sundberg A see Öbrink 49
- Ionnidou G B see Kalpaktsoglou 85
- Ireveld L see Hansson 181
- Jacobsen B see Falck Larsen 227
- Jafari K, Lash A F and Webster August D Pregravid and sarcoma 45
- Jensen H Changes in fetal supraventricular ectopic systoles during uterine contractions in labour 31
- Jerve F and Fylling P Therapeutic abortion—The 1978 report from Ullevål Hospital 237
- Johnsson J see Myers 317
- Johansson E D B see Axelsson 131
- Johnsson O see Moldin 179
- Johnsson J F see Frack 165
- Johnson J see Patch 381
- Jouppila P see Kuikka 749
- Kaar K see Kuikka 49
- Kallen B and Rybo G Conjoined twinning in Sweden 257
- Kalpaktsoglou P K, Ionnidou G B, Kondylis A I, Lekou S I, Papaconstantinou D and Christou A C Immunotherapy in adenocarcinoma of the ovary 85
- Karam K and Mroueh A Ovarian biopsy in the etiology of amenorrhea 701
- Kaskarelis D B An abdominal approach to the repair of post-hysterectomy vaginal inversion 13
- Kaskarelis D see Lohs 367
- Katz M see Sher 273
- Kaufmann H see Leiba 373
- van Kessel P H see Wallenberg 105
- Kjer J J see Gregersen 169
- Kjorstad K E see Welander 161
- Klebe J G see Christau 187
- Kobilková J see Štule 119
- Kolstad P see Welander 161
- Kondylis A P see Kalpaktsoglou 85
- Korsten C B see Frberg 761
- Kos L see Novak 95
- Kuikka J, Kaar K, Jouppila P, Pyörälä T and Rönkonen A An intravenous ¹²⁵I method for measuring regional distribution of placental blood flow 49
- Kullander S, Rauung A and Trupé C H Human ovarian tumours heterotransplanted to "nude" mice 143
- Kullander S see Persson suppl 78
- Ladachoff P and Maruszczak M A pregnancy with hydatidiform mole, thyrotoxicosis and live born infant 477
- Lahteenmäki P Influence of oral contraceptive use on immediate postpartum pituitary-ovarian function 76
- Lamke B see Stålen 435
- Lammintausta R, Erkkola R and Eronen M Effect of chlorothalidate treatment on renin-aldosterone system during pregnancy 289
- Lancet M see Djani 195
- Landberg T see Frack 165
- Larsson B see Patch 381
- Larsson B see Olund 333
- Larsson-Cohn U see Hammar 189
- Lash A see Jafari 45
- Laufe L see Ragab

- Lefter N see Beyth 469
 Ler S see Birle 349
 Le S Kaufman H Winkelsberg G and Bahary C Pregnancy in a case of Nelson's syndrome 373
 Lew S I see Kalpaktsoglou 85
 Libral H see Biale 483
 Loberg B S see Axelsson 113
 Lofstrom K and Ulmsten U Some methodological aspects on the measurement of intraluminal pressure in the female urogenital tract in vivo 63
 Lofgren A see Moberg 415
 Lofgren O see Salvatore 89
 Lofstrand T see Beckman 1
 Lof D and Kaskarelis D Human placental lactogen levels in amniotic fluid in normal and toxemic pregnancies 367
 Lofberg P see Bergman 373
 Ludrom Givika Treatment of primary dysmenorrhea with prostaglandin synthetase inhibitors—a promising therapeutic alternative 471
 Lutz D J see Shackleton 411
 Laitinen M Myometrial energy metabolism during pregnancy and normal dysfunctional labor suppl 71
 Lohu J M and Andersen H T On the use of epidural anesthesia in obstetrics 191
 Lofstrom Margit see Falck Larsen 777
 Lofgren K see Persson suppl 78
 Lofgren V J Jr Bygdeman M and Eneroth P The influence of locally administered prostaglandin E₂ and F on uterine motility in the intact non pregnant human uterus 141
 Lofszek M see Ladehoff 477
 Lof B Bar Am A and Delgidish Liane Cervical adenocarcinoma and partial hydatidiform mole 273
 Lof O Nygren A-G Fagerlund Christina and Hartvig P Absorption of a 8-hydroxy-quinoline (Steroids) through the vaginal mucosa 287
 Lofberg P J Eneroth P Hartin J Ljung Åsa and Nord C F Preoperative cervical microbial flora and post-abortion infection 413
 Loberg P J see Hansson 183
 Lofchuk M Honda T Deguchi M Monikawa H and Tojo S A study on the effect of dehydroepiandrosterone sulfate on so-called cervical ripening 397
 Lofgren P and Johansson O Acute fatty liver of pregnancy with disseminated intravascular coagulation 179
 Lof B R Hansen I T Diedrich P and Oram V Therapeutic abortion in an out patient clinic 41
 Lof B R Hansen I T and Fommensen S Effect of general and local anaesthesia on blood loss during and after therapeutic abortion 133
 Lofgren S see Moller 133
 Lofgren H see Michizuki 397
 Lof A see Karam 301
 Lof R E Johansson I and Adamsons K The effects of isotoproterenol on fetal oxygenation 317
 Lof R Hashiba N Washio M and Tojo S Human follicular apparatus and hormonal parameters in patients with primary and secondary amenorrhea 93
 Lof Karen see Vachavnikova 69
 Lof B and Behle I Perinatal mortality in hypertensive pregnant patients Its reduction in a developing country 19
 Nilsson B A see Axelsson 113
 Nord C E see Moberg 415
 Novak F Kos L and Pleiko F The advantages of the artificial vagina derived from sigmoid colon 95
 Nygren U-G see Meinik 287
 Öbrink A Bunne G Ulmsten U and Ingelman Sundberg A Urethral pressure profile before during and after pubococcygeal repair for stress incontinence 49
 Öbrink A see Bunne 335
 Olesen K P and Walter S Bladder base insufficiency 463
 Olund A and Larsson B Comparison of extra amniotic instillation of rivanol and PCF₂ either separately or in combination followed by oxytocin for second trimester abortion 333
 Oram V see Moller 41
 Papaconstantinou D see Kalpaktsoglou 85
 Patek E and Johnson I Recurrent hydatiform mole Report of a case with five recurrences 381
 Patek Eva and Larsson B Caesarean section A clinical study with special reference to the increasing section rate 745
 Pedersen H see Gaede 03
 Pehrsson P H see Gennser
 Perlman M see Sadoway 177
 Persijn J P see Enberg 761
 Persitz A see Deligdisch 439
 Persson P H Grenner L and Gennser G Ultra sound screening in pregnancy Methodology and significance suppl 78
 Plesko F see Novak 95
 Pulkkinen M O Salmunen J and Virtanen S Serum vitamin B₁₂ in pure pregnancy depression 471
 Pyorala T see Kuikka 749
 Pystynen P see Ikonen 741
 Ragab M I Edelman D A and Laufe L The effects of longacting paracervical block anesthesia on the abortifacient efficacy of intra amniotic PGF₂ and hypertonic saline 377
 Ramsøe Jacobsen J see Valenius 407
 Rao R see Bienenr 25
 Rausing A see Kullander 349
 Rekonen A see Kuikka 749
 Rolschau J Aspects of placental pathology and growth retardation A prospective patho-anatomical and cellular study suppl 72
 Rosengren E see Thorbert 43
 Rothe D J Cederqvist L L Zervoudakis A and Fuchs F Organization of amniocentesis for antenatal genetic diagnosis 7
 Rud T Ulmsten U and Andersson K E Initiation of voiding in healthy women and those with stress incontinence 457
 Ruponen S and Tama E Operative treatment of rectal endometriosis 277
 Rybo G see Kallen 257
 Ryden G The value of serum cystine aminopeptidase (CAPI) human chorionic somatomammotropin (HCS) and urinary oestrogen assays for detecting intrauterine growth retardation 211

- Ryden G and Berg G CAP HCS and urinary oestrol assays in diabetic pregnancy 313
- Rydner T see Bergqvist 177
- Sadovsky F and Perlman M Decreased fetal movements and polyhydramnios 177
- Salminen J see Pulkkinen 471
- Salvatore C A and Lodovico O Vaginal agenesis. An analysis of ninety cases 89
- Sandahl B Seasonal birth pattern in Sweden in relation to birth order and maternal age 393
- Scommegna A see Bieniarz 25
- Secher N J and Wallin L Haemodynamic effects of oxytocin (Syntocinon[®]) and methyl ergometrine (Methergin[®]) on the systemic and pulmonary circulations of pregnant anaesthetized women 97
- Shackleton C H L Macrae D J and Wilmott M P Comparison of oestrol in mother and fetus during labour and in the baby at birth 411
- Shih N See Bieniarz 25
- Sher G and Katz M Midtrimester intra amniotic administration of prostaglandin $F_{2\alpha}$ in combination with an hyperosmolar urea solution. Effect upon plasma levels of estradiol, progesterone and human placental lactogen (HPL) 223
- Snorraddottir M see Fieck 165
- Sorensen S An electroimmuno-assay of the pregnancy specific β_2 glycoprotein (SP₂) in normal and pathological pregnancies and its clinical value compared to human chorionic somato mammatotropin (HCS) 193
- Staland B Treatment of menopausal oestrogen deficiency symptoms in hysterectomized women by means of 17 β oestradiol pellet implants 781
- Sternthal Phyllis M see Bakkeberg suppl 77
- Stånge L Carlstrom K and Enksson Margareta Hypervitaminosis A in early human pregnancy and malformations of the central nervous system 749
- Sindbry J see Štule 125
- Štule J Švihovec J Drabková J Stihbny J Kobilkova J Vido I and Doležal A Electrical potential difference across the mid term human placenta 125
- Švihovec J see Štule 125
- Taina E see Ruponen 77
- Tatra G see Husslein 73
- Thorbert G Alm I and Rosengren F Cytochrome P-450 steroid induced changes in adrenocortical steroid level of Guinea pig uterus 45
- Tojo S see Mochizuki 797
- Tojo S see Nakano 793
- Trolle D see Gaede 701
- Tropé C see Kullander 149
- Tuimala R Chemical studies on lecithin (phosphatidylcholine) 74
- Ulmsten U see Lind from 13
- Ulmsten U see Rud 457
- Ulmsten U see Åkerlund 47
- Ulmsten U see Öbner 49
- Václavínková Vlasta Hedman Anna Kristina and Nyström Karen Follow up studies in dyplasia and carcinoma in situ of the cervix uteri 69
- Václavínková Vlasta see Bergqvist 177
- Václavínková Vlasta see Fianu 371
- Valerus N H and Ramoé Jacobson J Intra uterine supraventricular tachycardia 407
- Wallenburg H C van Kessel P H and Brand A van der Transfer of ⁵¹Cr platelets and ⁵¹Chromium hexa azotous the term rhesus monkey placenta 101
- Wallin I see Secher 97
- Walzer S see Olesen 461
- Washio M see Nakano 793
- Webster Augusta see Jafan 766
- Welander C Hjerstad K E and Holm P Preoperative irradiation and chemotherapy in patients with advanced ovarian cancer 161
- Vido I see Štule 125
- Wilmott M P see Shackleton 411
- Wingerup L Andersson K F and Ulmsten U Regulation of the uterine cervix and induction of labour at term with prostaglandin E_2 in viscous gel 401
- Winkelberg G see Leifu 373
- Virtanen S see Pulkkinen 471
- Wood C Diagnostic and therapeutic implications of intrapartum fetal pH measurements 13
- Yedwab G see Deligdisch 419
- Youssefnejadian F see Coats 171
- Zervoudakis A see Rasthe 7

